BIOLOGICAL BULLETIN

126762

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

Editorial Staff.

E. G. CONKLIN—*The University of Pennsylvania.*

JACQUES LOEB—*The University of California.*

T. H. MORGAN—*Bryn Mawr College.*

W. M. WHEELER—*American Museum of Natural History.*

C. O. WHITMAN—*The University of Chicago.*

E. B. WILSON—*Columbia University.*

Managing Editor.

FRANK R. LILLIE—*The University of Chicago.*

VOLUME V

WOODS HOLL, MASS.
JUNE, 1903, TO NOVEMBER, 1903.

Press of
The New Era Printing Company,
Lancaster, Pa.

CONTENTS OF VOL. V.

No. 1. JUNE, 1903

PAGE.

AXEL LEONARD MELANDER AND CHARLES THOMAS BRUES: *Guests and Parasites of the Burrowing Bee Halictus*..... 1

J. B. JOHNSTON: *The Origin of the Heart Endothelium in Amphibia* 28

J. W. SCOTT: *Periods of Susceptibility in the Differentiation of Unfertilized Eggs of Amphitrite*..... 35

ARTHUR W. GREELEY: *Further Studies on the Effect of Variations in the Temperature on Animal Tissues*..... 42

BENNET M. ALLEN: *The Embryonic Development of the Ovary and Testis of the Mammalia (Preliminary Account)*..... 55

No. 2. JULY, 1903

HENRY LESLIE OSBORN: *Bunodera cornuta sp. nov.: A New Parasite from the Crayfish and Certain Fishes of Lake Chautauqua, N. Y.*..... 63

J. B. JOHNSTON: *On the Blood Vessels, their Valves, and the Course of the Blood in Lumbricus*..... 74

VERNON L. KELLOGG: *Two New Genera of Mallophaga*..... 85

FRANK R. LILLIE: *Experimental Studies on the Development of the Organs in the Embryo of the Fowl (Gallus domesticus)* 92

W. C. CURTIS: *Crossobothrium laciniatum and Developmental Stimuli in the Cestoda*..... 125

No. 3. AUGUST, 1903

S. J. HUNTER: *On the Conditions Governing the Production of Artificial Parthenogenesis in Arbacia*..... 143

ESTHER F. BYRNES: *Heterogony and Variation in some of the Copepoda of Long Island*..... 152

CASWELL GRAVE: *On the Occurrence among Echinoderms of Larvæ with Cilia Arranged in Transverse Rings, with a Suggestion as to their Significance*.. 165

No. 4. September, 1903

Maulsby W. Blackman: *The Spermatogenesis of the Myriapods.* II. *On the Chromatin in the Spermatocytes of Scolopendra heros* 187
Helen Dean King: *The Effects of Heat on the Development of the Toad's Egg*..... 218
Thomas H. Montgomery, Jr.: *On Floscularia conklini, nov. spec., with a Key for the Identification of the Known Species of the Genus*..... 233

No. 5. October, 1903

C. M. Child: *Form Regulation in Cerianthus*..... . 239
Effa Funk Muhse: *The Eyes of the Blind Vertebrates of North America.* VI. *The Eyes of Typhlops Lumbricalis (Linnæus), a Blind Snake from Cuba* 261
Annie E. Pritchett: *Some Experiments in Feeding Lizards with Protectively Colored Insects*..... 271
S. J. Holmes: *Sex Recognition Among Amphipods*..... 288
T. H. Morgan: *Regeneration of the Leg of Amphiuma means*... 293

No. 6. November, 1903

H. F. Thacher: *Absorption of the Hydranth in Hydroid Polyps*.. 297
C. M. Child: *Form Regulation in Cerianthus*..... 304
Adele M. Fielde: *Artificial Mixed Nests of Ants*..... 320
Adele M. Fielde: *A Cause of Feud between Ants of the Same Species Living in Different Communities*..... 326
J. F. Garber: *Dimorphism in Blissus leucopterus.* 330
Raymond Pearl: *On two Cases of Muscular Abnormality in the Cat.* 336

Vol. V. *June, 1903.* *No. 1*

BIOLOGICAL BULLETIN.

GUESTS AND PARASITES OF THE BURROWING BEE HALICTUS.

AXEL LEONARD MELANDER AND CHARLES THOMAS BRUES.

During the months of summer every roadside presents a field of busy insect-activity, as varied and interesting as it is unseen and unheeded. Those insects, however, that we do notice are seen during their idling moments and hence we are generally accustomed to stigmatize all as idlers with no aim beyond song or frolic. But insects have a busy life — another phase of their existence which many of us overlook. If we inspect some roadside more attentively we shall be surprised to see many of the self-same idlers working with diligence. Spurred by parental anxiety these insects excavate their nests and store them with food, doing for their young what their parents have done for them.

Out of this multiplicity of insect-life we shall select as an example one of the burrowing bees of the genus *Halictus*, and endeavor to tell what may be seen on any summer day. *Halictus* (*Chloralictus*) *pruinosus* Robertson is a brilliant greenish bee, measuring about one third of an inch in length, which lives over an extended range, occurring from New Mexico, through Illinois, to Massachusetts. It is the commonest Halictine at Woods Hole, in the last-mentioned state, where the following observations were made. During the early part of summer these bees commence their excavations along the roadsides wherever a sandy slope presents a favorable situation, and continue their activities until early autumn, the colonies increasing in size, and becoming more closely settled as the season advances. They seem to be in the height of their vigor during the early part of September in this region. Although their social instincts are not so highly developed as those of *Apis* or *Bombus*, these bees

depart in their habits from the strictly solitary bees in that a male and two or three females are generally necessary for the successful direction of a single ménage. Moreover, a large number of nests are usually associated as a colony which may be scattered over a considerable distance or so populous that the tunnels almost intersect by their irregularities. The openings to the nests, however, are always separated by a distance of two or three inches or more. It can thus be readily seen that *Halictus* lives under conditions more or less similar to those of their more gregarious relatives, the ants, and hence it is not surprising that they are forced to harbor the same class of guests, and to be exposed to the same vicissitudes as are their cousins.

In constructing their nests the bees dig by means of their mandibles in the sandy clay, forming a hole of a diameter only slightly greater than will admit the largest female. The wall is then banked up with a plaster formed by the aid of saliva. Immediately behind the entrance is a short blind passageway, only large enough to allow a bee to turn on itself within.

This niche, which is always less than an inch from the entrance, serves simply to allow the bees to pass one another in the interior of the nest. From this point the gallery extends nearly straight back into the hill side, for a distance of a few inches and then slopes downward to the end — a total length of a foot or so. Near the further end jut a number of small diverticula radially extending from the main tunnel.

These are the nurseries of the young bees, where are stored the pollen and honey which is destined to serve as food for the bee larvæ of the coming generation. The excavation of the tunnels is a matter of considerable toil, requiring many days for its completion, but so industriously do the little bees work that at the close of day a miniature mound of sand has accumulated on the hill-slope below the opening. During the warm portions of the day the site of each colony of nests is a scene of inspiring activity. The air is filled with an ever-changing swarm of bees, each bent on its own task of excavation or of collecting honey and pollen, while from the openings of completed nests others can be seen peering about and eying everything that comes within their range of perception. At night everything is quiet, the trespas-

sers and robbers, too, have ceased their work, and the colony slumbers in peace.

The structure of the nest was ascertained by the ingenious plaster-cast method advocated by Prof. J. B. Smith. By this means the galleries of *Halictus* are seen to depart but little from those of the other burrowing bees. A passage-way for exit and entrance in addition to the regular one opening on the dumping ground, such as is constructed by *Augochlora humeralis* Patton,[1] was never noticed in the case of *H. pruinosus*, the vigilance required to guard two openings having probably prevented such an extravagance. All the burrows which we dug out, a dozen or so in number, extended in a nearly horizontal direction, and were always built on the very steep slopes along the roadsides. By

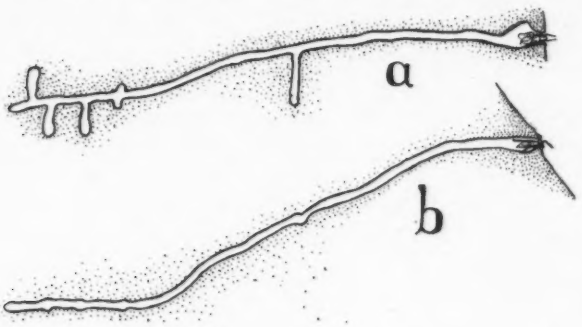


Fig. 1. Diagram of *Halictus* nest. *a*, plan; *b*, elevation.

this means none of the excavated dirt accumulated about the doorway, which was even cleared of all débris with but little effort on the part of the bee. The relatives of *pruinosus* in Texas, morphologically of the same species, select a level spot for their nesting-site, dig vertical burrows, and place the accumulated dirt in an irregular cone about the opening. A photograph of these nests is given for comparison.

During the latter part of nest-construction when the pollen has been gathered and the eggs laid, their home is continually threatened by thieves and kidnappers against whom a guarded watchfulness must be maintained. The sentinels are generally the

[1] J. B. Smith, *Proc. Am. Ass. Adv. Sci.*, 1898, p. 368.

males, who sit at the doorway, their rounded heads neatly filling out the entrance. When the female returns pollen-laden, the little guard slips into the first side passage while she enters, and then as quickly returns to his post. The incomers are perceived at a distance of half a foot, probably announced by the buzzing of their wings. Even when the little watchers can not see the female coming they dart half way out of their retreat at her approach. With antennæ vibrating and mandibles spread the males either manifest a joyful greeting for their nest-mates or show an

FIG. 2. Nest of *Halictus* near Austin, Texas.

equal degree of hostility towards any stranger that may venture too near.

The most dreaded of the enemies of the *Halicti* is perhaps the little velvet ant, *Mutilla canadensis* Blake, which is common nearly everywhere in North America, running about on the nests of these bees, its distribution practically coinciding with that of this species. Perhaps it is the stridulation produced by the abdomen of these intruders that arouses the ire of the guard at the door, for no sooner does one approach a nest than the watcher, if it be a female, rushes out and pounces upon the *Mutilla*, endeavoring to sting it to death. Down the hill-slope they roll, heedless of everything but an inborn desire to annihilate each

other. The *Mutilla*, too, is armed with a powerful sting, half the length of her abdomen, but the sagacious *Halictus* grasps her enemy about the waist and most successfully evades the sharp thrusts. These combats continue for many minutes, concluded either by the invulnerable *Mutilla* slipping from the bee's grasp, for her body is hard and sleek, or by the death of the more plucky *Halictus*. Each colony, where everything seemingly is peace and content, is thus turned into a field of carnage, with the bodies of one or more females ruthlessly tumbled to the bottom of the hill. If the bee escapes unscathed, which happily is the more usual outcome of these struggles, she spends a few moments in preening her body, and then returns to her nest. But no

FIG. 3. Nest of *Halictus* at Woods Holl, Mass.

greeting awaits her after her loyal struggle. When she hurriedly left the nest the male waiting his turn in the tunnel below quickly took her place as guard at the door, and now he blocks the entrance as obstinately as though it were a stranger begging admittance. The taint of *Mutilla* is still to be recognized on the body of the female and probably overpowers her family smell. For quite a minute she must remain at the door parleying with her mate before he is convinced of her identity.

This observation is of interest when considering the organic dependence of instinct. Fear of *Mutilla* has been cultivated

through natural selection and heredity till it manifests itself in the actions just recorded. But the conduct of the male towards his nest-mate, an inhospitable act which a gleam of reasoning intelligence would not permit under the circumstances, lends itself rather to the theory of a mechanical instinct, actuated in this case by the chemical nature of *Mutilla's* poison. If this be so it will be questioned why the bee does not behave as when *Mutilla* itself approaches. Does the mixture of *Mutilla*-influence and *Halictus*-influence compel an impassive head-on greeting while

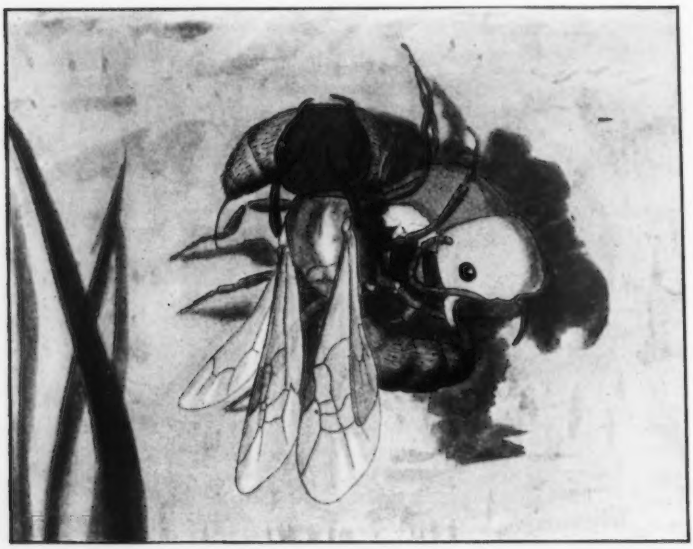


FIG. 4. Combat between *Mutilla* and *Halictus*. "Down the hill they roll heedless of everything but an inborn desire to annihilate each other."

Mutilla alone induces the male-watcher to turn tail in the manner described on the next page?

One little bee once displayed an originality not noticed again. For fully twenty minutes she had waited at the entrance of her home, gently urging admission by advancing to the nest-opening once each minute. The male would retreat a short distance each time but not sufficiently far to admit the female, who would then retire, resting with her antennæ almost touching those of the

stubborn gate-keeper. Finally she turned about and crept backward to the male, resting a moment with her sting before his face. When she now turned, the male seemed convinced, and the wearied female entered in the usual way. In this case did the female flaunt her own poison to overcome that of *Mutilla* as a passport to her home? It might seem so; but the simplicity of such a physiological action is quite equalled by the complexity of the intelligence displayed.

When a male bee guards the opening the approach of *Mutilla* produces a far different effect upon the watcher. Instead of rushing out on the marauder, the defenseless male adopts the less foolhardy measure of "turning tail," but still keeps at the entrance of the nest. Now the convex abdomen neatly fits the opening, forming a parasitic-proof shield, and *Mutilla* must needs leave. When no other bee is behind a female watcher, she never rushes out, leaving the nest unguarded, but adopts a manœuvre similar to the male's, but instead of inflexibly curving her abdomen over the opening, she reaches afar with her sting.

Canadensis, however, is not the only Mutillid that worries the Halictines. On numerous occasions *Myrmosa unicolor* Say[1] and *Mutilla infensa* sp. nov. were found crawling about, but these species do not appear to have become nearly so annoying. From one square meter of *Halictus*-colony fully fifty specimens of *canadensis* were taken during the summer, whereas in all but ten specimens of the *Myrmosa* were observed. *Mutilla ferrugata* Fabr. and *vesta* Cresson were also found prowling over the nests, though these species are doubtless parasitic on the larger burrowing insects which associate with *Halictus*, for the large size of their bodies would not permit entrance into the *Halictus* nests. Moreover, they may crawl quite close to the doorkeeper and elicit no attention; possibly their stridulation is pitched to an unresponsive key and their odor stimulates no reaction.

Almost as ardent a persecutor of the bees is to be found in a

[1] It is time to abandon superfluous names. *Myrmosa unicolor* Say, described as a male, and *M. thoracica* Blake, described as a female, have paraded in collections quite long enough as distinct species. Inasmuch as Mr. H. L. Viereck has recently taken the initiative (*Ent. News*, 1902, p. 72) in consolidating some of the species of Mutillidæ, we shall follow him in the nomenclature of this paper. The males of this species fly abundantly among the roadside flowers, in company with males of *canadensis* and *ferrugata* (= *castor* Blake = *Lepeletierii* Fox [*fenestrata* Lepeletier]).

new species of *Phora*.[1] This little fly takes a stand near an opening and patiently awaits an unguarded moment. Then she quickly slips in to deposit an egg in the pollen so industriously stored. One *Phora* persisted in her attempts to enter for several hours. Driven back a half inch by the doorkeeper she gradually and slowly returned until she nearly touched his face. Then a sudden lunge half way out of the nest on the part of the bee would drive her back again. This was repeated over and over, the doggedness of the parasite and her slow approach seeming to exasperate the little watcher. By turning his head he tried to follow her movements, but from their very slowness was unable to discern her position. Only when his palpi were touched would he make a sudden dart. *Phora* depends on her agility as well as on her deliberateness. On each return of the female bee, after a fifteen-minute foraging trip, the parasite would jump about excitedly and possibly would get a chance to oviposit on the pollen mass during a dart at the bee. A moment's rest on the threshold would grant the nervous little fly ample time to infect the unsuspecting bee. The behavior of the bees towards *Phora* is quite different from the action of ants towards these guests. Unless irritated by the persistence of the parasite, *Halictus* is passive and does not notice its presence. Even the incoming females do not see the fly at a distance of half an inch. On the other hand, ants are put in a state of fright by the proximity of these flies. During the attacks of the ant-decapitating phorid, *Apocephalus Pergandei* Coq. upon the species of *Camponotus, ferruginea* in the north, and *maculatus* var. *sansabeanus* in the south, the ants rush in the wildest excitement with wide-spread mandibles at the agile fly. Can this difference result from the bees never seeing their offspring and being consequently unaware of their fate, whereas the ants have a personal acquaintance with the ravages of these parasites? It might seem so, but we must remember that in the case of *Pachycondyla harpax*, at least, a phorid larva is not only tolerated in the nest, but is also fed by its host.[2] In this case, however, no harm is done to the species by the presence of the fly, whereas with *Halictus* it must mean the death of the brood.

[1] *P. halictorum*, described in the sequel.

[2] Wheeler, W. M., *Am. Nat.*, 1901, p. 1007 *et seq.*

The most conspicuous of the smaller Hymenoptera that frequent these grounds is a little species of *Loxotropa*. Time and again this insect was observed crawling stealthily over the nest-colony, tapping its antennæ on the ground as it moved. During this deliberate progress it covers an inch in four seconds, but as soon as its nears a selected opening its movement slows down to an almost imperceptible advance. Still holding its long and clubbed antennæ extended straight forward, their tapping now reduced to a slight nervous vibration, it gradually insinuates itself into the nest, even beneath the very jaws of the gatekeeper. Often after crawling so far into the nest that only the tip of its abdomen is visible, it finds the nest unsuitable. Then it deliberates no longer, but makes a hasty exit, leaving the astonished

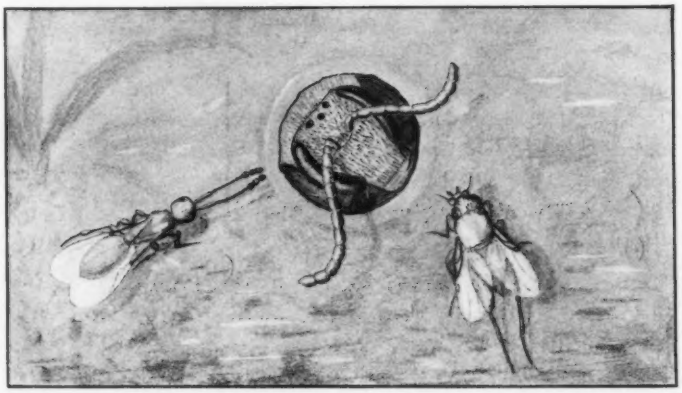


FIG. 5. *Loxotropa ruficornis* Ashm. *Halictus*, ♂. *Phora cata*, sp. nov.

sentinel to reach in vain with questioning antennæ for its bold and impudent disturber.

As interested an observer of the incoming bees as is the *Phora*, is a tachinid fly. This species hovers over the breeding ground and suddenly circles over a particular hole. Is it attracted to the nest by the hollowness of the sound of its vibrating wings as it flies over an opening, or does it discern the state of advancement of the household below by an instinct less mechanical? Like its relatives, this species chooses the moment when the incoming bee pauses at her threshold quickly and quietly to oviposit on her pollen mass and thus infect her offspring.

A number of ants, foragers from near-by nests, are always to be found on the nesting-ground. These belong to harmless species which do not molest the bees. When an ant and a bee meet on the nest there is no encounter, each retreating good-naturedly to go her own way. The Stenammas, especially, have a stridulatory note as plaintive as that of *Mutilla*, yet this is unnoticed by the bees; even the watchers rest unaroused in their doorways while the ants pass them by. The little red thief ant is also found nesting in the midst of the bee-colony. Evidently it is here to ply its vocation of tunnelling into the chambers of the bees to steal from them their honey.

The little beetle, *Bæocera concolor*, seems quite at home with the bees. Although it belongs to a family of fungus-beetles, it, nevertheless, must have some intimate connection with the bees, as it was repeatedly observed running familiarly in and out of the nests. It is quite possible that it may live upon the pollen in deserted nests which has become mouldy by the growth of fungus hyphæ. The mixture of pollen and honey is thus readily turned into a mass of fungus under certain conditions.

The woes of the Halicti are not yet at an end. Another insect is as persevering in its depredations as its colleagues, and accomplishes by boldness what the others try by stealth. This is a larger foe, *Philanthus punctatus* by name, which audaciously builds its nest in the center of the *Halictus* colony, and when ready swoops down on a bee, stings it to death, and carries it home. Not one but many bees meet this death at the sting of their unsuspected neighbor, who plans her murders so that they take place at the flowers where the bees are at work.

When we consider the persistence of the Mutillas we can appreciate the extent to which specialization in keeping the nest parasite-proof has been carried by this bee. Seldom are the entrances left unguarded, and never is a stranger bee granted admission. In this respect *Halictus* is far more conservative than the wasp *Trypoxylon*. Although mistakes in selecting their own domicile from a cluster of fifty similar nests were frequently made, the watchers always recognized these visitors as strangers and were instantly ready to show fight. *Trypoxylon*, a wasp which also guards its doorways, on the contrary, makes no ob-

jection to the free entrance of visitors of the other sex, as has been shown by the Peckhams.[1] *Mutilla canadensis* appears to be the most dreaded enemy, as it alone is noticed by the bees. With a little reasoning ability many of the other parasites could be readily annihilated, whereas no move is made for protection against these foes except by the guard at the door. But how are the bees to know, even in the case of *Mutilla*, that their guests mean harm to their progeny? Probably they do not in a strict sense. It is evident, however, that the instinct of guarding the entrance to the nest could have been developed through the

FIG. 6. *Stethopathus occidentalis*, sp. nov., lateral view.

action of natural selection of favorable variations in habit, while it would be difficult to derive a number of specific reactions towards the different guests in the same manner. The very commonness of *Mutilla* and its conspicuous size are probably the reason that a specific reaction has been developed in this single case. *Halictus* is far less sensitive to its surroundings than many of the fossorial wasps are, coming and going even though we dis-

[1] "Instincts and Habits of the Solitary Wasps," p. 79, 1897.

turbed the nest and remained close by. Its one fear is centered in *Mutilla*. With thief-ants to rob its nests, parasites to prey on its offspring, and in constant danger of being carried away bodily by a wasp, itself numerous in individuals, it is remarkable that *Halictus* should have become a dominating type throughout such a wide territory.

This ends the list of the enemies of the bees as we have observed them. Many other insects abound on the nesting-site, but most of these, at least, are accidental visitors which neither harm nor are harmed. Several beetles, spiders, flies and other insects are included in this list which we give for reference in conclusion. The smaller species live near the *Halictus* as they would do anywhere, and not through preference, and the larger ones in part are attracted to our observation ground to prey on the smaller. These transients are such as a careful observation of any limited field would bring to notice. They are the participants in life's continual struggle, each seriously and unwittingly playing its part.

PART TWO.

A List of the Insects, Including the Accidental Visitors, Found About the Colonies of Halictus pruinosus, Robertson, at Woods Hole, Mass. July–August, 1902.

Class ARACHNIDA.

Epeirid sp.

A minute larval spider was several times seen. It has no connection with the *Halictus*.

Bathyphantes formica Emerton.

Quite a number of specimens of this strange spider were observed running in their zigzag course over the ground. Like the last it is an accidental visitor, occurring on the colony during its search for food. We are indebted to Mr. Nathan Banks for the determination of this species.

Acarina spp.

Two species of mites were obtained, one of which (*Bryobia pratensis* Garm. ?) occurred in numbers.

Class MYRIAPODA, DIPLOPODA.

Polyxenes fasciculatus Say.

Numerous specimens found crawling about on the sand.

Class INSECTA.

Order **Thysanura.**

The genus *Podura*, represented by many specimens, was found associated with the former.

Order **Hemiptera.**

Aleurodes sp.

The larval form of an Aleyrodid was discovered on the nest. Probably it is that of *A. corni* Hald., the commonest form of the Atlantic States.

Order **Diptera.**

Family CHIRONOMIDÆ.

Ceratopogon hollensis sp. nov.

Third vein in part confluent with the first, ending much beyond the middle of the wing, wings in large part hairy, not uniform in coloration, but not spotted; eyes well separated; tarsal claws simple, of an equal length; legs not spinose beneath; metatarsus much longer than the second tarsal joint.

Female. — Head fuscous, proboscis black. Antennæ fuscous, the joints uniformly moniliform, slightly longer than broad, the last two joints longer. Eyes widely separated, the front yellowish. Mesonotum pruinose, sparsely and uniformly covered with short black bristles. Abdomen dark fuscous, lightly gray pruinose, apically hairy. Pleuræ paler fuscous, smooth. Halteres dark fuscous, the stems paler. Legs slender, uniformly yellowish, except that the knees, and the tips of the femoral and tarsal joints are very narrowly black; tibiæ provided with several simple long but slender hairs on the outer edge; no bristles below, tarsi somewhat hairy, claws small, uniform, simple, empodium small. Wings sparsely covered with short bristle-like hairs, more or less serially arranged. These become obsolete at the very base, cinerascent with a pale brown tinge becoming stronger along the basal part of the course of the anterior heavy veins, gradually interrupted in front of the anterior cross-vein, then gradually recommencing

to end abruptly before the tip of the first vein. The crotch of the furcation of the light vein crossing the anterior cross-vein is darkened by an accumulation of pigment and by an increase in the number of hairs.

Length, 0.85 mm.

Woods Hole, Massachusetts, August, 1902.

The nearest relative of this species is *C. variipennis* Coq.

It is not unlikely that the species is an halictophile, as it was several times seen upon the nests, thus suggesting its myrmecophilous relatives. It may also be the cause of the presence of some of the proctotrypidæ here listed, as some of them are known to prey on the larvæ of various species of the genus. This is the case with *Adeliopria* Ashm., a Diapriid, which is parasitic on a Texan species of *Ceratopogon*.[1]

Family MYCETOPHILIDÆ.

Sciara sp.

A *Sciara* would frequently fly over the nesting-site and alight on the open ground. It is an accidental visitor more at home in the nearby grass.

Family PHORIDÆ.

Phora halictorum sp. nov.

Female.—Length, 1.5–2.25 mm. Head black, subshining, antennæ black; palpi dull yellow, with stiff black bristles below; proboscis not exserted; front long, flattened, punctured, shining, its bristles reduced in size, and those of the middle row placed high up. Anterior four proclinate bristles small, the remaining ones placed normally.

Thorax black, subshining, the dorsum finely pubescent, the pleuræ lightly pruinose, ten bristles present on the hind edge of the mesonotum, dorsum with one pair of dorsocentral and four marginal scutellar bristles.

Abdomen black, shining though not brilliant, not bristly, lightly pruinose basally along the sides; ovipositor short, retractile, piceous.

Legs piceous, front legs somewhat lighter, front coxæ dull yellowish, middle and hind coxæ piceous, hind coxæ with the usual ridge on the posterior side; hind femora stoutest, twice as thick as the front ones, middle femora intermediate, all the tibiæ with short bristles, biserially arranged on their outer side, those of the front tibiæ ten to twelve in number and approximated into one line towards the inner forward edge, those of the other tibiæ in two separated series, for the middle tibiæ four in the outer and six in the inner row, and for the hind tibiæ seven in the outer and ten in the inner rows; front tibiæ without terminal spurs, middle tibiæ with one

[1] See Wm. H. Ashmead, BIOL. BULL., 1902, p. 15.

long spur three fourths the length of the metatarsus, hind tibiæ with two moderately long spurs, the outer one two-thirds as long as the inner, which is nearly as long as that of the middle tibia.

Wings hyaline with faint cinereous tinge, not brilliantly iridescent, the heavy veins nearly black, reaching very nearly to the middle of the wing. First vein but slightly bowed, third vein nearly straight, furcate, costal bristles fine and short, thickly placed, distributed as follows: four proximal to the humeral cross vein, twenty-two (double series) bordering the costal cell, ten (double series) bordering the marginal cell, and six (in double series) along the submarginal, *i. e.*, the furcation of the second heavy vein. Thin veins dark, the fourth longitudinal slightly flexed only at its extreme base, so that the cell in front is slightly wider than the one behind, ending a little closer to the wing-tip than the second light vein does, seventh vein evident, extending into the wing-margin. Halteres whitish, their stem dusky.

Male.—Length, 1.75 mm. Differs as follows: frontal bristles stouter, abdomen smaller, genitalia not distinct, small, the central filament fleshy, short, directed backward. Tibial setulæ and the inner spur of the hind tibiæ reduced in size, ridge of the hind coxæ large; costal bristles not uniform, disposed thus: prehumeral four, twenty along costal cell, four along marginal cell and four along the submarginal. The inner bristles are minute, becoming larger at the third pair of the costal cell, and from thence are much stronger than in the female.

Described from several specimens, collected as described in the previous account at Woods Hole, Mass., July–August.

This species is related to *agarici* Lintner[1] but differs by the longer bristles on the tibiæ, longer front, four scutellar bristles, etc. The habits also are quite different, as *agarici* feeds upon decaying mushrooms.

Phora rostrata sp. nov.

Female.—Length, 1.5–1.75 mm. Black, shining, legs more or less yellow, lower frontal bristles proclinate, third vein forked.

Head shining black, especially smooth and polished on the front and vertex. Front with the normal chætotaxy except that there are only two proclinate bristles at the lower edge. The front is also sparsely hairy besides the large bristles. Median longitudinal groove and ocellar tubercle unusually well-marked. Antennæ black, with a distinctly plumose arista. Proboscis piceous, very large and strongly exserted, as long as the head-height. It is slender at the base where the rather small bristly spindle-shaped black palpi are inserted, then much enlarged, swollen and bifurcated at the extremity. The bifurcation is produced by a splitting of the apex by a horizontal slit in the proboscis. Thoracic dorsum shining, hairy as usual,

[1] 10th N. Y. Rept., pp. 399–406.

with one pair of dorsocentral and two scutellar bristles. Abdomen black, nowhere bristly. Legs pale yellow, the tarsi sometimes brownish; hind tibiæ very indistinctly ciliated and with a single weak spur, as have also the middle pair. On the inner side at the apex the posterior pair have several transverse rows of short black bristles. Wings yellowish hyaline, the costal vein reaching distinctly beyond the middle of the wing and with very short cilia. First vein ending a little closer to the tip of the second than to the humeral cross vein. Fourth vein evenly arcuate, fifth vein sinuate as is also the sixth; seventh vein present. Halteres yellowish, blackened at the tips.

Described from two female specimens collected at Woods Hole, Mass., July 15, 1902, about the burrows of *Halictus pruinosus*.

This species is readily recognizable on account of the excessive development of the proboscis, which is evidently adapted to some peculiar method of food-getting. It is also characterized especially by the very shining front, which seems to place it near to the European *P. minor* Zett., with which it agrees is some other characters.

Phora cata sp. nov.

Male and Female.—0.8–1.2 mm. Black, legs and palpi yellowish or brown, antennæ of male enlarged. Anterior frontal bristles proclinate.

Head black, front short, about as wide as long, subshining, faintly gray pollinose in the male, two anterior bristles proclinate, the others all present and arranged as usual. Antennæ wholly black in the male, in which sex the third joint is enlarged and ovate so as to be very conspicuous, in the female they are of the usual size and slightly yellowish at the base; arista pubescent. Palpi light yellow, strongly bristly. Proboscis of female projecting, stout and horny. Thorax shining, black, hairy, with one pair of dorsocentrals and two marginal scutellar bristles. Abdomen black. Legs yellowish-brown, the anterior pair lighter. Posterior femora ciliated below on apical half, their tibiæ without any rows of small bristles on the outer side; four posterior tibiæ each with a delicate apical spur. Wings hyaline, the costal vein not quite reaching to the middle of the wing, its cilia short and closely placed. Third vein far from the costa at its base, and forked very near the apex. Tip of first vein twice as far from the humeral vein as from the tip of the second. Fourth vein slightly but evenly curved, recurved at the extreme tip. Fifth slightly diverging from the fourth to its tip, which is as far behind the wing tip as the fourth is before it. Seventh vein faint but distinct. Halteres yellowish in the female, piceous in the male.

Described from a single pair from Woods Hole, Mass. The lighter color of the female is most likely due to her apparently

immature condition. They were taken on the sand in the midst of a colony of *Halictus*.

This species can readily be recognized in the male sex by the enlarged third joint of the antenna. The female is not so characteristic, but can be distinguished by the combination of structural characters given in the description. It resembles most closely *P. agarici* Lintner, but has very short costal bristles.

Stethopathus Wand.

Among the insects frequenting the ground immediately about the *Halictus*-burrows was one extremely small form, which from its quick motions we immediately suspected to be a wingless phorid fly. Such it indeed proved to be, but of quite a different sort from any of our previously discovered North American species. Its occurrence in New England is quite unexpected and considerably extends the range of such forms, as none have hitherto been seen in America north of central Texas.

Its associations with the *Halictus* may be doubtful, although no specimens could be found elsewhere whereas three females were captured where the burrows of the bees were abundant. Nests of *Lasius niger* and of *Stenamma fulvum*, variety *piceum* also abound in such locations, but close scrutiny of the ant nests revealed no specimens of the Phoridæ. The fact that species of *Phora* occur as parasites of these bees would make it seem not improbable that the *Stethopathus* has similar habits. We have also a single winged male phorid, captured at the same time, but which is probably the male of some other undescribed form on account of its larger size and the different chætotaxy of the head. The description of this interesting little wingless fly, one of the smallest known of all the Diptera, is given herewith.

Stethopathus occidentalis sp. nov.

Female.—Head rounded triangular, much rounded on the sides and at the hind angles and obtusely pointed in front, about two thirds as long as wide above, vertex descending rather steeply and evenly. Eyes small, about one and one third times as large as the second antennal joint, coarsely facetted with hemispherical ommatidia as usual. Antennæ placed at the bottom of the deep frontal cavities. Proboscis long and stout, equal to the head-height ; palpi small and slender, thickest near the tips, with stout macrochætæ on the inner side. Ocelli present, placed in a small

triangle on the vertex. Head with four closely approximated macrochætæ at the middle of the front margin, two widely-separated ones near the anterior corner of the eye directed inwards and two outwardly directed ones near the posterior angles ; a series of small macrochætæ below and in front of the eye.

Thorax small, twice as wide as long, truncate before and behind ; sinuate on the sides and narrowed behind, so that the pleuræ are slightly visible from above. Thorax rather sharply arched above, and much narrowed below on the sides. Dorsal surface with a pair of long macrochætæ just

FIG. 7. *Stethopathus occidentalis* sp. nov., dorsal view.

behind the anterior angles and four smaller marginal ones along the posterior edge.

Abdomen considerably swollen, but with large and strongly chitinized dorsal plates. The first is only a narrow band, contiguous with the second which is very large and contiguous with the third. The fourth and fifth are separated by a white membrane such as covers the abdomen elsewhere. Seen from above the abdomen is twice as wide as the thorax and

flattened, oval in cross-section. No ventral sclerites are present. Each segment is margined behind with small bristles and is hairy elsewhere as is the entire body. Glandular opening of the fifth segment[1] in the shape of an arcuate slit. External genital organs of the usual form. Legs rather stout, the tibiæ with two apical spurs.

Length, 0.75 mm. Testaceous, head and thorax darker above, especially directly about the ocelli. Abdominal plates dark fuscous, the membranous parts almost white, with a small fuscous spot at the insertion of each hair.

This form is a typical representative of the Stethopathinæ and strange to say, it approaches more nearly to the East Indian *Stethopathus ocellatus* Wand. than to any of the species that have hitherto been discovered in America. Indeed, it is here regarded as congeneric with the former, although the two species are from such widely separated regions of the earth.[2] It may be necessary later to separate these two forms, but at the present state of our knowledge of this group it does not seem advisable. The American species resembles *S. ocellatus* Wand. in possessing ocelli, being utterly destitute of wings and halteres, and in having a similarly shaped head and abdomen. But differences in form are also evident: the thorax is only twice as wide as long, instead of three times as in *ocellatus*, the palpi are clavate, not spindle-shaped, and the chætotaxy is somewhat different, although conforming to the same general type. Although its habitus seems to be quite different from that of the genus *Ænigmatias* Meinert (which it may be recalled, has just been discovered in Arizona,[3] a locality quite distant from its home in Denmark), yet this species may possibly prove to be a close relative..

A point perhaps of minor importance, but nevertheless interesting as bearing upon its systematic position, is the fact that the

[1] In previous papers the gland opening has been referred to the fourth segment of the abdomen, but the very short first segment in the present species leads us to believe that this sclerite is concealed in the other American species and that there, too, the gland really opens on the fifth segment.

[2] Many cases might be mentioned of monotypical or very small genera of insects which have an inexplicably wide discontinuous distribution. *Amphizoa* with two species, one in western North America and another in Tibet; *Syntelia*, which is represented by two species, occurs in Mexico and eastern Asia; and the water-beetle, *Pelobius*, occurring in western Europe, Tibet and Australia. For further references to the close approximation in certain details of the faunæ of eastern North America and Asia, see C. C. Adams, "The Southeastern United States as a Center of Geographical Distribution of Flora and Fauna," BIOL. BULL., Vol. III., pp. 115, et seq.

[3] D. W. Coquillett, *Can. Ent.*, 1903, p. 20.

American species has, like the East Indian form, bare, non-pubescent macrochætæ, while the other American species of this subfamily have them pubescent.

Family TACHINIDÆ.

Metopia leucocephala Rossi.

The interested observer of the *Halictus* mentioned in the first part was captured for identification, and proves to belong to this widely distributed species.

Order **Hymenoptera**.

Family BRACONIDÆ.

Subfamily CHELONINÆ.

Chelonus brevipennis sp. nov.

Female.—Length, 2 mm. Ferruginous, head piceous black, wings reaching just beyond the base of the abdomen.[1] Antennæ 21-jointed, tapering as usual, and almost as long as the body, ferruginous at the base, black at the tip, the third joint four times as long as thick, the apical joints more or less quadrate-moniliform. Eyes smaller and less densely hairy than usual. Head almost smooth above, shining, thinly pale pubescent, piceous black above, ferruginous below, palpi yellow. Thorax ferruginous, pronotum coarsely rugose reticulate above, mesonotum less distinctly so, metanotum small, quadrate, not toothed at the posterior angles, rugoso-reticulate; pleuræ not roughly sculptured, somewhat shining. Abdomen with no traces of sutures above, dark ferruginous and sparsely white hairy; gradually broadened from the base and rounded at apex; finely and irregularly reticulately striate longitudinally, especially at the base. The incurved margin is emarginate at the apex of the abdomen. Ovipositor stout, black. Legs long and slender, yellow, the femora clavate.

Described from a single female specimen collected at Woods Hole, Mass., in a burrow of *Halictus pruinosus*.

The present species seems best referable to *Chelonus* because of its pubescent eyes. The apex of the abdomen however is emarginate, somewhat as in *Gastrotheca* Guérin. Unfortunately as the wings are rudimentary they can not be used to determine its affinities. The only other apterous species belonging to this subfamily are included in *Acampsis* Wesmael, from which the present form differs by its unsegmented abdomen.

[1] For neuration, see BIOL. BULL., 1903, p. 189, Fig. 5.

Family CHALCIDIDÆ.[1]

Eupelmus rhizophelus Ashmead.[2]

This remarkable chalcidid with vestigial wings in the female was seen rather commonly about the *Halictus* burrows. As it has been previously bred from cynipid root galls by Mr. Ashmead, it is no doubt an accidental visitor to the bee nests.

Eupelmus Ashmeadii sp. nov.

Female. — Length, 3.5–4 mm., ovipositor 0.5 mm. Shining green varied with ferruginous on the thorax and with luteous and black on the abdomen. Head shining green, with a sparse white pubescence. Mandibles brown, black at the tips, palpi black. Antennæ long, the scape yellow, reaching to the ocelli, flagellum black, about once and one half the head-height, last joint acutely pointed. Head less than twice as wide as long, the space between the eyes above narrow, so that the lateral ocelli are close to the eye-margin. Face rugoso-punctate with a median carina extending from the clypeus to the insertion of the antennæ. Prothorax shining brown. Mesonotum very closely punctate, not at all shining, brown in front and green behind, concave medially behind, on each side of the depression it is raised and almost carinate, then slopes down to the reflexed margin; anteriorly it is raised to form a broad triangular tubercle. Pleuræ ferruginous except in front where they are green. Metanotum golden, closely punctate, bilobed, sharply declivous, forming a right angle with the mesonotum. Wings deeply infuscated, paler at base and slightly so at apex, with a narrow cross band of white just before the stigmal vein. Marginal vein equal to one third the length of the wing, stigmal vein moderate, one half the length of the post-marginal. Abdomen shining black, pale luteous on the basal third. Sheaths of the ovipositor bright ferruginous, almost as long as the abdomen. Legs brown, darker on the front and hind femora, tarsi yellowish except the tips.

Described from three female specimens collected at Woods Hole, Mass., July and August, 1902.

This pretty species was associated with the much smaller brachypterous species, *Eupelmus rhizophelus* Ashm., on the burrows of *Halictus pruinosus*. It is named in honor of Mr. Wm. H. Ashmead, who determined it as an undescribed species.

Henicopygus subapterus Ashmead.

We have seen this species running actively about on the ground among *Halictus* burrows at Austin, Texas. Like the species of *Eupelmus*, it may be an accidental visitor.

[1] We are indebted to Mr. Wm. H. Ashmead for his kindness in determining the species of Chalcididæ.

[2] For wing-neuration, see BIOL. BULL., 1903, p. 189, Fig. 7.

Encyrtinæ gen. et sp. indesc.

Among the Chalcididæ there is a single specimen which Mr. Ashmead, who has kindly examined it, informs us represents an undescribed genus of Encyrtinæ. Unfortunately it is too poorly preserved to permit of an accurate characterization in the large and difficult group to which it belongs.

Cirrospiloideus (Miotropis) platynotæ Howard.

A single female of this species was captured.

Superfamily PROCTOTRYPOIDEA.

Family SCELIONIDÆ.

Telenomus sp.

There is a single pair representing an apparently undescribed species in this large and difficult genus.

Caloteleia Marlattii Ashmead.

This active little species is a regular visitor about the nests.

Caloteleia parvipennis sp. nov.

Female. — Length, 2.5 mm. Yellow, varied with darker. Head black, very smooth and polished above the antennæ, finely punctured on the vertex and with larger punctures intermixed. Mandibles yellow at the base, black at the tip. Antennal scape pale yellow, reaching a little above the vertex, the pedicel small and rounded, yellow, the flagellum about one and one half times the length on the scape, black, the first flagellar joint twice as long as the pedicel, then the joints decrease in size to the fourth, the following six forming a thick oval club with closely articulated joints. Thorax entirely yellow, except the tegulæ which are black, mesonotum finely punctulate, with two rather faintly marked furrows, scutellum large, semicircular, smooth. Metathorax very short, emarginate in the middle, smooth on the sides. Abdomen polished and perfectly smooth, except for coarse longitudinal striæ on the first and at the base of the second segments. The petiole is short quadrate, and bears a quite distinct polished black tubercular horn at its base ; basal half of abdomen otherwise yellowish varied with brown, apical half black ; third segment longest, second nearly as long, others much shorter. Legs including the coxæ yellow. Wings short, reaching only to the middle of the abdomen. Marginal vein short and swollen, stigmal about one third as long as the lengthened postmarginal, costal margin sparsely ciliated.

Described from one female specimen taken at Woods Hole, Mass., on a slope that was thickly riddled with the burrows of

Halictus. When captured it made no attempt to fly, the wings evidently being too much atrophied to be of functional use.

This form can be readily recognized by its short wings. It does not seem to be very closely related to any of the other North American species.

Scelio ovivorus Riley.

This large and coarsely sculptured Scelionid was originally bred by Scudder from the eggs of the common New England grasshopper (*Dissosteira carolina*) so that its occurrence is evidently not connected with the presence of the *Halictus* colony. Nevertheless it was often seen intermingling with the bees.

Family DIAPRIIDÆ.

Loxotropa ruficornis Ashmead.

This is a common species always to be found on the breeding ground of these bees. Its habits have already been noted in the preceding part of this paper.

Family BETHYLIDÆ.

Empyris subapterus sp. nov.

Female.—Length, 3.25 mm. Black, head and thorax subopaque, abdomen shining; antennæ, mandibles at tips, palpi, tegulæ and extreme tip of abdomen rufous; sparsely pale pubescent. Head about one third longer than wide, closely and finely punctate with fewer larger punctures intermixed. Antennæ reaching about to the tegulæ, scape stout and curved, three times as long as its thickness at the tip; following joints of about equal length, except the first flagellar, which is shorter; pedicel more slender, the other joints slightly wider than long. Eyes hairy, ocelli present. Prothorax sculptured like the head, with a transverse impressed line anteriorly. Mesonotum very short, less than half as long as wide, without grooves or furrows. Tegulæ rufous. Scutellum basally with a deep transverse linear fovea. Metanotum about one and one half times as long as wide, with a median longitudinal carina and a fainter one close on each side of it anteriorly, also a lateral and an apical carina present; surface elsewhere finely transversely rugulose; posterior face sharply declivous, shining and punctulate. Wings abbreviated,[1] just attaining the apex of the metanotum; with a small stigma near the apex, a narrow, submarginal cell and an equally long but wider basal cell; costal margin fringed. Legs, including the coxæ, dull rufous. Abdomen polished black, the margin of the penultimate segment and the apical half of the last segment ferruginous.

[1] For figure see BIOL. BULL., 1903, p. 189, Fig. 2.

Described from several female specimens collected at Woods Holl, Mass., running about among the burrows of a colony of *Halictus pruinosus* Robts.

This species greatly resembles *Mesitius* in habitus, but has a transverse furrow at the base of the scutellum instead of two foveæ. It can hardly be the undescribed female of *E. carbonarius* Ashmead, on account of the difference in the sculpture of the metanotum. It is apparently the first subapterous form to be described in this genus.

Family FORMICIDÆ.

Lasius niger Linneus.

Stenamma fulvum var. **piceum** Buckley.

Solenopsis molesta Say.

This last named species is the only one that derives any direct benefit from the presence of the bees.

Family MUTILLIDÆ.

Mutilla canadensis Blake.

This is the most conspicuous of the enemies of the bees. It has been fully noticed in the preceding part.

Mutilla infensa sp. nov.

Female.—Clothed with sparse appressed white pubescence becoming denser apically, and with scattered long erect hairs. The hairs are black on the vertex, dorsulum and second abdominal segment and become whitish on the under side of the body and beyond the second segment of the abdomen. Coarsely sculptured species; head finely and closely punctate, thorax and petiole of the abdomen coarsely reticulate, abdomen much less deeply and more distantly punctured than the head, the apical segments with finer punctures, meso and metapleuræ shining, not or but little strigose, nearly smooth, pygidium longitudinally closely but irregularly striated, the striæ very weak and vanishing apically. Head quadrate, concave behind, in profile also rounded; eyes prominent, round, subshining, their facets distinct; mandibles straight, strong, pointed, untoothed; scape stout, as long as the three basal joints of the flagellum, basal flagellar joints subequal. Thorax elongate-oval, nearly as broad as the head, the front margin and angles well defined, posterior surface of the metanotum not sharply declivous, somewhat flattened and rounded above. Petiole of the abdomen flattened above, constricted from the second segment, one fourth broader than long, its front angles sharp and prominent, its ventral carina weak,

very obtusely angulate at the middle and minutely toothed in front. Legs slender, provided like the body with silvery erect hairs, four or more strong spines on the outer edge of the hind tibiæ, the tibial spurs and spines black.

Ferruginous or somewhat darker, the mandibles, the flagellum except its basal joint, *i. e.*, the third antennal joint, more or less of the second abdominal segment, and all of the other segments of the abdomen, from the third apically, both ventrally and dorsally black. Legs including the coxæ piceous or black. Second segment of the abdomen with a varying extent of the front margin, a diffused median vitta and the hind margin more strongly black or blackish. On each side of the median stripe is a pair of conspicuous rounded testaceous spots. Last ventral segment sometimes reddish.

Length, 4.75 mm.

Woods Hole, Massachusetts. Parasitic on *Halictus pruinosus* (?).

The edentate mandibles, the facetted eyes and the nodose petiole of the abdomen would lead one in placing this species in the small group *scrupea*, where it is obviously distinct from the only other known female by its rugose thorax, etc. Notwithstanding this, we shall have to disregard the well-marked ommatidia and place the species in the group *occidentalis*, intermediate between *cariniceps* Fox and *rugulosa* Fox, differing from each by the structure of the pygidium, etc., but related by its general habitus, sculpture and chætotaxy.

Mutilla vesta Cresson.

Mutilla ferrugata Fabricius.

Like the former species this too is doubtless parasitic on the larger Hymenoptera such as *Philanthus* or the Pompilidæ that nest near by.[1]

Myrmosa unicolor Say.

The males of this species fly about the roadside flowers while the females are frequently found about the bee nests. Their presence is undoubtedly due to the bees.

Family PHILANTHIDÆ.

Philantus punctatus Say.

This species was observed nesting in the very midst of several of the colonies of *Halictus*.

[1] In Europe Sichel records *M. incompleta* Lep. as parasitic on *Halictus* (cf. Horæ, *Soc. ent. Ross.*, VI., p. 11) and *M. coronata* as a parasite of *Larra anathema* (*ibid.*, p. 12).

Sphex ichneumonea Linn., and a species of Pompilidæ were also seen digging their nests in the compact sand of the road in the vicinity of the bee colony. They have no connection with the presence of the bees, but associate with them as the same condition of soil and surroundings are suitable for each.

Order **Coleoptera.**

Family COCCINELLIDÆ.

Microweisea misella Leconte.[1]

The species of this genus are reported to be of great economic importance as they greedily prey on scale insects. The presence of the *Aleyrodes* may have had an influence in bringing this species to our notice.

Family ENDOMYCHIDÆ.

Aphorista vittata Fabricius.

Family PTINIDÆ.

Cænocara scymnoides Leconte.

Family SCAPHIDIDÆ.

Bæocera concolor Fabricius.

The last three species are fungus-eating beetles, which may come to the *Halictus* nests to feed on the fungus overgrowing the stores of abandoned or damp nests. It is certain that during the course of the season numerous nests are left unfinished, either deserted voluntarily by the bees for some whimsical reason or not completed by the death of the bees.

Family RHIPIPHORIDÆ.

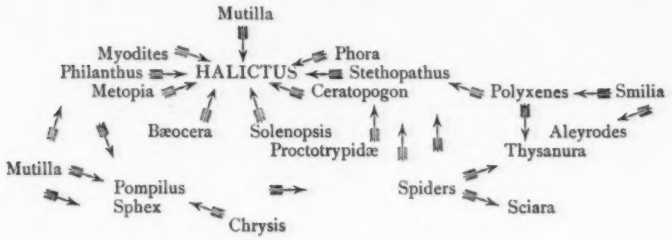
Myodites fasciatus Say.

Inasmuch as Fabre and others have found the larvæ and pupæ of a member of this family in the cells of an European species of *Halictus*, it is quite interesting to note the occurrence of *M. fasciatus* about the colonies of the American form. Several specimens were taken while sweeping with the net among the swarming bees as they entered and left their nests.

Several other beetles were found crawling over the nest but were visitants too accidental to record.

[1] This is the species known in our lists under the generic name *Smilia* or *Pentilia*. The present name was proposed by Cockerell (*Can. Ent.*, 1903, p. 38).

In conclusion we may present the following diagram showing the interrelationships of the most important of the insects we have observed. For *Halictus* it is indeed a "whirlpool of life" with only too many vortices centered upon its unfortunate self.



THE ORIGIN OF THE HEART ENDOTHELIUM IN AMPHIBIA.[1]

J. B. JOHNSTON.

The origin of the heart endothelium in Amphibia has been the subject of several special investigations and of a considerable volume of discussion. The question of fact may now be regarded as settled. The work of Brachet has given definite and conclusive evidence that the endothelium is derived directly from the entoblast, as had been shown to be very probable by the earlier work of Rabl and Schwink. The question now of interest is, how is the derivation of the heart endothelium from the entoblast in amphibia to be harmonized with its known origin from the mesoblast in all other vertebrates? The problem is that of the homology of the heart endothelium of amphibia. Granted that, as Ziegler contends, the condition in amphibia is to be regarded as the result of cœnogenetic modification, exactly what is the modification that has taken place? What is the definite explanation of the striking difference between amphibia and other vertebrates? As Brachet has pointed out, the term "cœnogenesis" can not be invoked as a magic symbol to dispense with the whole matter. It is not enough to say that in amphibia the endothelial cells remain connected with the entoblast until a late period and become separated after the mesoblast sheet has split off. This offers no escape from the difficulty pointed out by Morgan ('97, p. 151) that the heart endothelium must be considered to have a different origin from the rest of the heart.

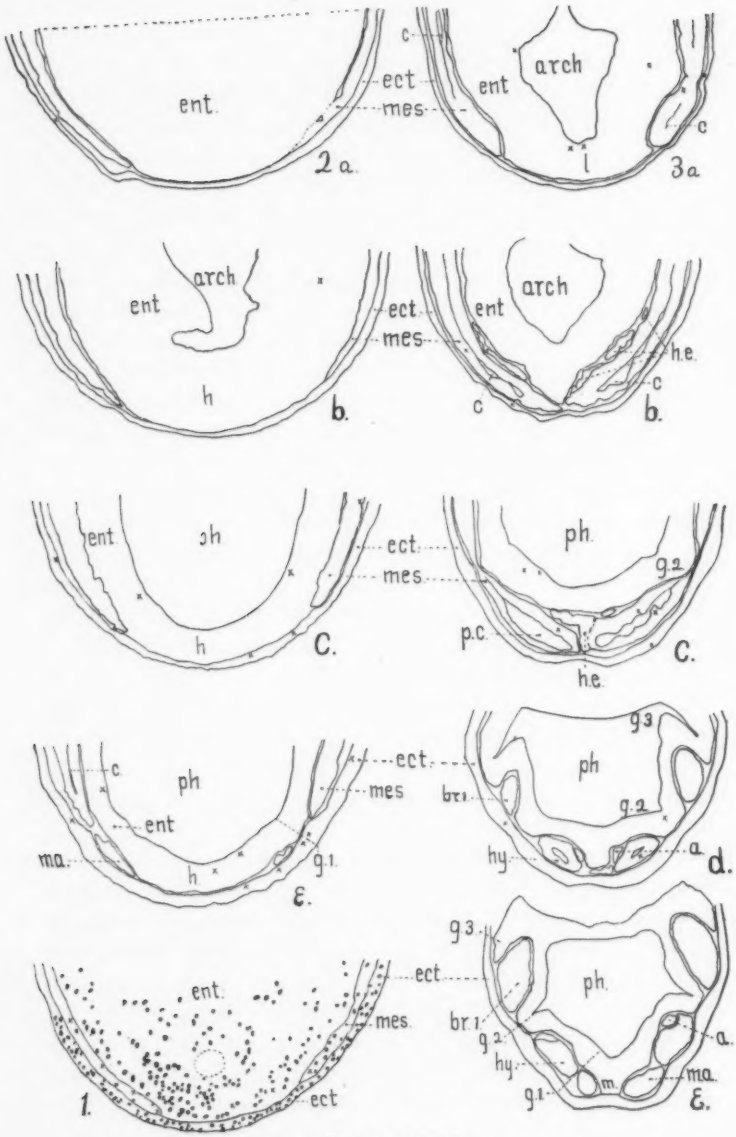
The work upon which the present paper is based has been done upon the eggs of a salamander which have been used for class study for the past two years. The species has not been identified because no adults have yet been taken. I hope at some later time to give a description of these eggs and to deal with some other features of the embryology of the species. The eggs

[1] Studies from the Zoölogical Laboratory of West Virginia University, No. 7 February 27, 1903.

have proved very favorable for study and the facts are so clearly made out that they are thought to offer a solution of the problem.

The earliest indication of the formation of the heart endothelium is found in the rapid multiplication of the cells of the entoblast just behind the mouth anlage, at a period when the head is slightly turned downward and before the gill slits have begun to appear. As shown in Fig. 1, the nuclei in this part of the entoblast are small, rounded, very numerous and closely crowded, and many of them are in some stage of mitosis. The nuclei in the remainder of the entoblast are larger and irregular, being much distorted by pressure of the yolk grains, and mitotic figures are rare. The area described extends for a considerable distance backward from the mouth, and the same conditions prevail on the cephalic surface and the sides of the pharynx close to the mouth anlage. Rapid growth in these latter regions continues later than behind the mouth and is connected with the formation of head mesenchyme. The region of growth behind the mouth is noticeable in both transverse and sagittal sections, but it is of short duration and in slightly later stages the cells are relatively larger and the nuclei have the appearance of resting nuclei. At the point nearest the mouth the cell divisions continue until the time of separation of the heart endothelium.

The formation of the mesoblast and its early differentiation furnish the facts of greatest significance for our problem. In the head and anterior part of the trunk the mesoblast is split off from the entoblast to a point some distance from the mid-ventral line, where the delamination appears to stop. That this is a definite limit beyond which delamination does not go is evidenced by the distinct separation between entoblast and mesoblast which often occurs even in very early stages (Fig. 2, *a* and *b*), by the total absence of nuclei in the outer half of the entoblast ventral to the limit mentioned, and by the future history of the ventral portion of the mesoblast. Mitotic figures often appear very early in the ventral edge of the mesoblast sheet (Fig. 1), and although they do not appear in the sections drawn in Fig. 2, they are usually more numerous there than elsewhere in the mesoblast. The result of rapid growth here is to cause a decided thickening of the ventral edge of the mesoblast, and in this thickening the body


ent.
ect
mes
2a.
c
arch
ent
l
c
3a.
ent
arch
ect
mes
h
b.
ent
arch
h.e.
c
c
b.
ent
oh
ect.
mes
h
C.
ph.
g.2
p.c
h.e.
C.
c
ph
ent
ect
mes
ma.
h
g.1
ε.
g.3
ph
br.1
g.2
hy
a
a
d.
ent.
ect
mes
ect
1.
g.3
ph.
br.1
g.2
hy
a.
m.
ma.
g.1
ε.

FIGS. 1, 2 AND 3.

cavity early makes its appearance (Fig. 3, *a*). With further growth the body cavity enlarges and the entoblast is laterally compressed between the cavities of the two sides. As a result, the growing entoblast behind the mouth, above described, takes on the form of a keel. Later the body cavity (pericardial cavity) spreads ventrally and mesially, and the mesoblast insinuates itself between the heart endothelial cells and the ectoblast and later between these cells and the entoblast. This movement is due entirely to the growth and spreading of the mesoblast earlier split off and not to a further delamination from the entoblast. There is no sign of any further delamination of mesoblast after the stage shown in Fig. 2, but on the contrary the mesoblast grows continually more and more sharply distinct from the entoblast after that period. The pushing down of the mesoblast in the region of the heart, which accompanies the enlargement of the pericardial cavities, is well advanced while the thickened ventral edge of the mesoblast farther caudally has not shifted its position (Fig. 3, *a*, *b*, *c*). The region in which the delamination of mesoblast does not reach the mid-ventral line extends caudally to a point a little behind the middle of the embryo and this region probably includes the blood island described by Brachet. The writer has not yet fully investigated this region, but if the surmise here made is correct, the reasoning applied to the question of the heart endothelium will apply equally well to the blood island. To recapitulate, there is a mid-ventral area or keel of entoblast extending backward from the mouth anlage, from which no mesoblast is split off in the species studied. From this area the heart endothelium (and perhaps the blood) are formed.

A second fact of some interest for us is that the mesoblast shows a tendency to split off late, so that it is already divided into regions when it first separates from the entoblast. This is seen especially in the formation of the mandibular arch. As shown by Fig. 2, *e*, the mandibular arch mesoblast, at its first appearance is separated from the rest of the mesoblast by the first gill slit, and it never has any connection with the mesoblast bounding the pericardial cavity. Indications of the second gill slit also appear very early, so that in some cases the hyoid arch, which is continuous with the pericardial cavity, seems to be split

off from the entoblast separately from the rest of the mesoblast. Finally, single cells wander off from the cephalic surface of the entoblast and go immediately to the formation of head mesenchyme.

The mode of formation of the heart endothelium from the ventral keel of entoblast differs in details in different forms. In the Urodeles studied by Brachet, the keel of entoblast extending from the mouth anlage to the region of the liver splits off as a continuous rod, the cells of which later arrange themselves into a tube. In the species studied by the writer the cells of this keel do not remain in a continuous rod but split off singly or in groups of a few cells and form a loose mass which remains connected with the entoblast longest at the end nearest the mouth. At this point there is continued growth and there is probably a migra-

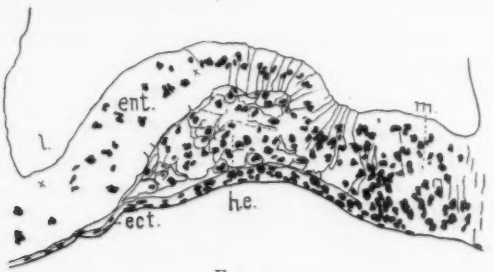


FIG. 4.

tion of cells from this point backward, and also upward into the several branchial arches, to form the aortic arches. The splitting off of this keel of entoblast is taking place simultaneously with the spreading ventrally and mesially of the pericardial mesoblast. My preparations leave no doubt whatever that the heart endothelium is formed from the most superficial portion of the entoblast in the mid-ventral region and that the lateral sheets of mesoblast are formed wholly outside of this area. Brachet's description makes it clear that the same thing is true of the Urodeles which he studied, but this important relation seems not to have attracted his attention and the fact is not mentioned by him.

We are now able to state definitely the nature of the cœnogenetic modifications connected with the formation of the heart endothelium in amphibia. According to the earlier accounts

which have recognized the derivation of the endothelium directly from the entoblast, the mesoblast sheets were split off first, and later — consequently from deeper layers of entoblast — the cells destined to form the heart endothelium were split off. Since in other vertebrates only one layer of cells is split off and the heart endothelium is differentiated from a part of this mesoblast, the conclusion that the endothelium of amphibia has a different origin from that of other vertebrates was unanswerable. In the species studied by the writer (and also, apparently, in those described by Brachet) the mesoblast sheets are split off earlier from the entoblast except in the region in which the heart endothelium will appear, and later the endothelium is split off from a part of the entoblast which has not given rise to any (other) mesoblast. Therefore, in these forms, the heart endothelium is derived from the same source as the mesoblast sheets, namely from the superficial layer of entoblast, and the difference between these amphibia and other classes of vertebrates consists only in a somewhat general tendency for the mesoblast to split off relatively late and to be marked out into definite organs, or organ-anlages, at the moment of splitting off. This is seen not only in the splitting off of the heart endothelium at a little later time and separately from the rest of the mesoblast, but also in the same mode of formation of the mandibular and hyoid arches and of a part of the head mesenchyme. The writer believes that a reëxamination of other amphibia, at least of Urodeles, at the proper stages of development will show the process here described to be characteristic for amphibia. In brief, then, the heart endothelium of amphibia is strictly mesoblastic, although it is not at any stage identified with the undifferentiated mesoblast, being split off from the entoblast in the same manner as the rest of the mesoblast, but somewhat later and separately.

DESCRIPTION OF FIGURES. ABBREVIATIONS.

a., aortic arch cells; *arch.*, archenteron; *br.1*, first branchial arch; *c.*, cœlome; *ect.*, ectoblast; *ent.*, entoblast; *g.1*, *g.2*, *g.3*, first, second, and third gill slits; *h.*, heart region; *h.e.*, heart endothelium; *hy.*, hyoid arch; *l.*, liver region; *m.*, site of mouth; *m.a.*, mandibular arch; *mes.*, mesoblast; *p.c.*, pericardial cavity; *ph.*, pharynx. Small crosses indicate the position of mitotic figures. In Figs. 1 and 4 resting nuclei are shown as black spots.

FIG. 1. Transverse section through the ventral part of a young embryo immediately behind the site of the mouth, to show the area of growth in the entoblast preparatory to the formation of the heart endothelium. The dotted circle indicates the position in which the foregut appears in the next section forward.

FIG. 2, *a*, *b*, *c*, *e*. Transverse sections nos. 212, 224, 235, 251 of an embryo in which the first gill slit has just made its appearance. Sections 10 microns thick.

FIG. 3, *a*, *b*, *c*, *d*, *e*. Transverse sections nos. 273, 292, 306, 318, 325 of a later embryo in which the separation of the endothelial cells from the entoblast is nearly completed. Sections 10 microns thick. The sections shown in Fig. 3, *a*, *b*, *c*, *e* are approximately at the same levels as those shown in Fig. 2,*a*,*b*,*c*,*e*, respectively.

FIG. 4. Median sagittal section of the region between the mouth and liver of an embryo of the same age as that shown in Fig. 3. Cell boundaries are shown wherever they can be seen. The heart endothelial cells are evidently continuous with the entoblast behind the mouth, but independent at all other points.

All figures were drawn with Zeiss apochromatic lenses and camera. Figures 1, 2, and 3 were drawn with 16 mm. objective and no. 4 ocular; Fig. 4 with 8 mm. objective and no. 4 ocular, and all have been reduced to one third in reproducing.

BIBLIOGRAPHY.

Brachet.

'98 Developpment du coeur chez les Amphibiens urodeles. Archives d'Anatomie micr., T. 2, p. 251–304, 1896.

Morgan.

'97 The Development of the Frog's Egg, an Introduction to Experimental Embryology. New York, 1897.

Ralb.

'86 Ueber die Bildung des Herzens der Amphibien. Morph. Jahrb., Bd. 12, pp. 252–273. 1886.

Schwink.

'91 Untersuchungen iber die Entwickelung des Endothels und der Blutkörperchen der Amphibien. Morph. Jahrb., Bd. 17, pp. 288–333. 1891.

Ziegler.

'02 Lehrbuch der vergleichenden Entwickelungsgeschichte der niederen Wirbeltiere. Jena, 1902.

PERIODS OF SUSCEPTIBILITY IN THE DIFFERENTIATION OF UNFERTILIZED EGGS OF AMPHITRITE.

J. W. SCOTT.

While studying the unfertilized egg of *Amphitrite* at the Marine Biological Laboratory, Wood's Holl, Mass., I verified Fischer's [1] result that the eggs could be caused to develop cilia by squirting them from a pipette, by transferring them from one dish to another, or by some other sort of mechanical agitation. I believe however that it is inadmissible to speak of this development as parthenogenesis, meaning the production of a normal embryo from an unfertilized egg. Ciliated, swimming structures result, but their differentiation takes place with only partial or abnormal and usually without any definite segmentation. I will discuss the morphology of these processes in another paper. Lillie [2] has shown clearly a similar differentiation in the *Chætopterus* egg.

In addition to Fischer's results, I found: (1) At least two critical periods in which the egg is highly susceptible to mechanical stimulation, one period thirty to forty-five minutes, the other eighty to one hundred minutes after they are removed from the body and placed in sea-water; (2) slight agitation is more effective in the second period than in the first; rougher handling is better in the first than in the second in which the eggs are more easily broken into fragments. (3) Frequent and moderate squirting after thirty to fifty minutes seems more effective than one hard squirting after the same time.

In my early experiments with certain salt solutions, the results were sometimes discrepant, and there was great variableness in the number of swimming eggs obtained under apparently identical conditions. About this time Fischer's paper came into my hands. He had shown that "parthenogenetic development can be produced by adding a small amount of Ca-salt to sea-water"

[1] Fischer, Martin H., *Am. Jour. Phys.*, 1902, III., p. 301.

[2] Lillie, F. R., *Archiv für Entwickelungsmechanik der Organismen*, 1902, XIV., p. 377.

and by "mechanical agitation." "The unfertilized eggs of *Amphitrite*," he says, "develop to the trochophore stage if, after residence in sea-water from one half to one hour, they be squirted from a small nozzled pipette into another dish of sea-water." "The method is an uncertain one," depending upon "state of ripeness," and a "previous residence in sea-water or in one of the sea-water-salt solution mixtures is essential." He had noticed that some eggs are very sensitive to "mechanical manipulation," but rarely develop when treated "immediately after they are cut out of the body of the animal."

Already convinced that there was a time-factor to be considered, I planned the following series of experiments. In each series a set of eggs, removed from a single female at the same time, was used. Due precautions were taken to prevent fertilization by previously washing the female thoroughly in fresh water. The hands of the operator, the dishes and pipettes used, were carefully sterilized in the same way. For the same reason, sea-water was used which had been raised to a temperature of 60° C., cooled and aërated. After washing in fresh water, the *Amphitrite* was placed in a dish of sterilized sea-water until the eggs were removed. In the following four experiments the eggs were removed from the female at 2.10 P. M. July 30, and were at once transferred very carefully to fresh sterilized sea-water.

Experiment 1.—The object of this experiment was to test the effect of transferring from one dish to another. In order to get a standard amount of agitation, the eggs were allowed to fall, one drop at a time, from the mouth of a pipette held one inch above the water. The different lots of eggs and the time each was transferred are given below:

1	control, transferred	2:10 P. M.
2	transferred	2:27 P. M.
3	"	2:43 P. M.
4	"	2:58 P. M.
5	"	3:13 P. M.
6	"	3:43 P. M.
7	"	4:13 P. M.
8	"	4:43 P. M.

The dishes containing the transferred eggs were left undisturbed until 10 P. M., when eggs were taken from 1, 2, 4, 6, 8

and examined. Care was taken to avoid disturbing those left in the dishes.

The control showed nearly all eggs unchanged; in a few the germinal vesicle had broken down and they were darker (more opaque) in color; a few had started to segment.

Lot 2. The germinal vesicle had broken down in nearly all; a "perivitelline space" found in about 20 per cent., but was rather small in most of this number. Most of the eggs were light (translucent) in color.

Lot 4. An irregular "perivitelline space" in 40–50 per cent. The germinal vesicle was broken down in practically all, the light-colored as well as the dark. There were a few extra-ovates.

Lot 6. The germinal vesicle broken down in nearly all; a "perivitelline space" in 40–50 per cent., irregular in some; a smaller number are blackened.

Lot 8. A prominent "perivitelline space" and contracted protoplasm in 50–60 per cent.; the rest have the germinal vesicle intact.

Amphitrite eggs frequently begin differentiation if left in seawater entirely undisturbed. This is shown in the above control. The experiment so far disclosed no marked phenomena, and I give the above descriptions to indicate the comparatively uniform development at this time. No evidence of normal segmentation was found at any time in this and the three following experiments.

All the dishes were again examined at 9:30 the next morning, as the advanced stages afford a better means of testing the effects of transference. Below is given the estimated number of ciliated eggs found in 2,000 of each lot. Aside from the swimming eggs, the different lots were in practically the same condition as on the previous evening. No further description is then necessary.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1	0 min.	0
2	17 "	0
3	33 "	10
4	48 "	10
5	63 "	4
6	93 "	60
7	123 "	10
8	153 "	10

Experiment 2. — The object of the experiment was to test the effect of a more violent method of transferring. The eggs were taken up in a pipette and squirted with moderate pressure into the dish of sterilized sea-water from a distance of two or three inches; then water in the dish was taken up three times and squirted at the surface. The control was simply transferred.

An examination of these eggs was made at 10:15 P. M., when their condition was not much different from those in Experiment 1, except that more showed effects of the agitation. The next morning, 10:15 A. M., the following results were obtained:

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	7 "	2
3	23 "	10
4	38 "	40
5	55 "	0
6	70 "	3
7	100 "	20
8	130 "	4
9	160 "	0

Experiment 3. — Eggs were transferred in the following lots and squirted moderately as in Experiment 2. Thereafter they were squirted again moderately at frequent (10–15 min.) intervals, up to 4:40 P. M. Examined at 9 A. M. July 31.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	32 "	60
3	60 "	0
4	120 "	4

Experiment 4. — The eggs were squirted violently, the water vigorously agitated by squirting with a pipette, and then left undisturbed.

Condition at 8:45 A. M., July 31. Many fragments present.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	33 "	40
3	60 "	2
4	120 "	0

I have taken the above experiments as typical examples. I have occasionally obtained a much larger per cent. of swimming eggs, frequently a smaller number, and sometimes none. Accepting the number of swimming structures as a fair test of development of this kind, we may make again the following statements:

1. In the differentiation of unfertilized eggs of *Amphitrite*, produced by transference, squirting or other methods of agitation, there are at least two periods in which they are highly susceptible, one thirty to forty-five minutes, the other eighty to one hundred minutes after being put into sea-water.

I have attempted to depict this idea on ordinate paper, shown in the accompanying figures. Abscissas represent time from the beginning of an experiment, ordinates the relative number of

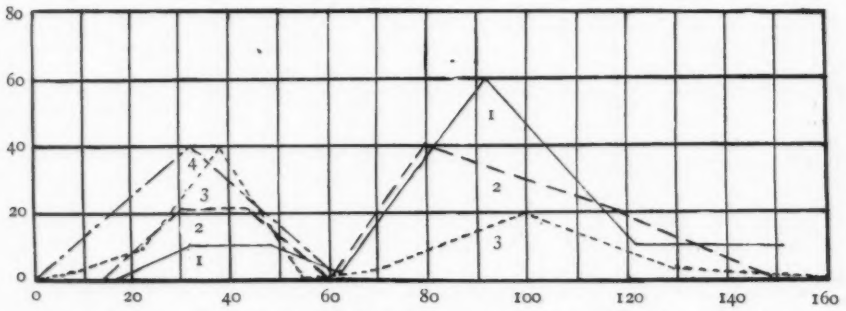


FIG. 1. 1. Gently transferred, experiment 1. 2. Very moderately squirted, experiment 26. 3. Moderately squirted, experiment 2. 4. Violently squirted, experiment 4.

swimming eggs in 2,000 of each lot. Fig. 1 gives the results of four experiments produced by different degrees of agitation. Fig. 2 shows all the observations of these four experiments combined in a single line; where two observations were made at the same time their average is taken (in one case only). The dotted line gives my idea of the curve of susceptibility, as brought about by a moderate degree of shaking.

2. By comparing experiments 1, 2 and 3 (Figs. 1, 1, 2, 3) we find slight agitation is more effective in the second period than in the first; rougher treatment causes more to develop in the first period, but injures some in the second.

3. Frequent and moderate squirting after thirty to forty-five minutes seems more effective than one hard squirting, after the same time. Compare experiments 3 and 4.

A comparison with fertilized eggs is of interest. The normal egg throws off the first polar body in less than thirty minutes after fertilization, and the first cleavage appears about thirty minutes later. According to Loeb's[1] view, the sperm in the case of parthenogenetic eggs acts simply to hasten, or accelerate, processes which are already present in the egg. It has frequently been noticed that the unfertilized egg of *Amphitrite*, if left undisturbed in sea-water, will often show some phenomena of differentiation. Assuming Loeb's theory as a working hypothesis, we should expect artificial means to be slower than fertilization. This proves to be the case; fertilized eggs develop cilia sooner

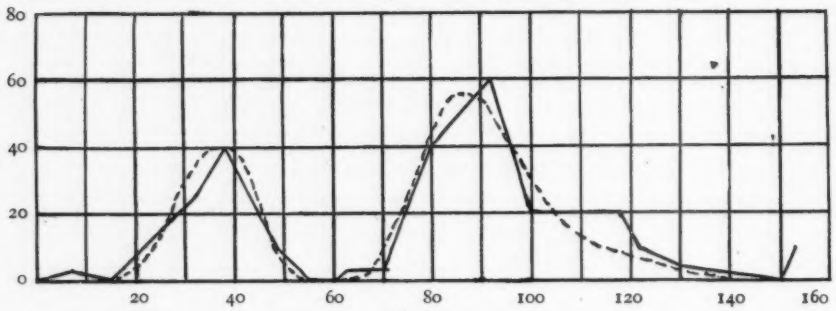


FIG. 2.

than those squirted. Sometimes there is not much difference in *Amphitrite*. Presumably, then the two critical periods mentioned correspond to processes in the normal egg that are active about the time for the appearance of the first polar body and the first cleavage; there is the same relative time between them. Further work is needed to prove this. Delage[2] states that the starfish egg is highly susceptible to "artificial fertilization" between the breaking down of the germinal vesicle and the appearance of the first polar body.

However this may be, it is certain that *there are processes of differentiation going on in the unfertilized eggs of Amphitrite which*

[1] Loeb, J., *Am. Jour. of Phys.*, 1901, Vol. IV., No. IX.

[2] Delage, Y., *Archiv d. Zool. Exper. et. Gen.*, 1901, T. IX., Nos. 2–3.

may be started into activity at definite intervals by mechanical agitation. These processes are, for the most part at least, independent of the processes that cause segmentation. I have noticed, as a rule, that the riper the eggs are the more cleavage is found, but I am convinced that it is never normal beyond the first few segmentations, if at all. It would seem, then, that the sperm introduces the active cause of this process.

It has been shown by Lyon[1] in the fertilized *Arbacia* egg, that there are recurring periods of susceptibility to KNC poisoning, and to lack of oxygen. Each period of susceptibility is followed by a period of resistance. On the other hand, in the unfertilized eggs of *Amphitrite*, there are at certain times unstable conditions, during which a small amount of agitation will set these unstable forces free, and lead to some definite characteristics of more advanced development (*i. e.*, production of cilia, etc.).

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO.

[1] Lyon, E. P., *Am. Jour. of Phys.*, 1902, Vol. VII., No. 1.

FURTHER STUDIES ON THE EFFECT OF VARIATIONS IN THE TEMPERATURE ON ANIMAL TISSUES.

ARTHUR W. GREELEY.

This paper contains an account of a series of experiments which are the outcome of others of a similar nature described in two earlier papers by the same author.[1] This previous work has called attention to the fact, noted by other observers, that the fluidity of the protoplasm of any of the Protozoa studied, varies directly with the temperature up to a certain critical point (28°–35° C.), above which the protoplasm suddenly goes into heat rigor, or coagulates. My own work has shown that as the temperature is lowered below the normal, a similar coagulation sets in which causes the cell to lose water.[2] This loss of water and coagulation is accompanied by a gradual cessation of the vital activities of the cell, and brings about certain very definite morphological changes that result in the formation of resting cells, which consist only of an undifferentiated mass of protoplasm. In the case of *Monas*, these changes were carried further by exposing the cells to a still greater reduction in the temperature, and the resting cells were finally broken up into many small spores, each of which reproduced the motile organism when returned to the normal temperature. As the temperature is raised above the normal, the protoplasm takes up water and all its vital activities are accelerated, until coagulation suddenly ensues at the critical point.

[1] Greeley, *American Journal of Physiology*, 1901, VI., p. 122. BIOLOGICAL BULLETIN, 1902, III., p. 165.

[2] This fact that lowering the temperature to 1° to 5° C. and raising the temperature above the critical point has the same effect upon protoplasm (*i. e.*, coagulation and loss of water), has received an interesting verification in the recent work of Fischer on Lepidoptera (*All. Zeit. für Entomologie*, October 15, 1901). In experiments on the artificial production of seasonal varieties of *Vanessa anteopa* by exposing the larvæ to different degrees of temperature, Fischer discovered that precisely the same variations in the adult forms are produced by lowering the temperature to 1° C. or raising it to 40° C., while modifications in the temperature within those limits gave strikingly different results.

In order to determine whether similar structural changes, as have already been described in the cases of *Stentor* and *Monas*, could be produced on other forms as well, the low temperature experiments have been continued on many other Protozoa, both Infusoria and Rhizopoda, and in all of them changes identical with those described above have been obtained. *Monas* is the only form in which it has been found possible to control the formation of spores, but in all the others resting cells were formed at the low temperature, which reverted to the motile condition when restored to the normal temperature.

I. The Reversal of Vital Phenomena by a Reduction of the Temperature.

The results of these low-temperature experiments on the Protozoa suggested an interesting comparison to the experiment of Loeb's,[1] in which the tentacles and polyps of a Campanularian Hydroid were reduced to the undifferentiated protoplasm of the stolon by bringing them in contact with some foreign substance. It appeared that for the Protozoa a lowering of the temperature as well as a contact stimulus brings about just such a reversal of the vital phenomena until the undifferentiated resting cell is formed, while a small increase in temperature accelerates the metabolic processes. To see if a lowering of the temperature brought about similar changes in the more complex multicellular animals a series of experiments was begun on the fresh-water Hydra.

It was at once observed that whenever a Hydra is exposed to a temperature of 4° to 6° C., the tentacles gradually become shorter and thicker, and are finally completely absorbed into the body. As the absorption goes on, the ectoderm and entoderm cells of the tentacles lose their individuality and form an undifferentiated mass of protoplasm, which is slowly taken into the body of the Hydra (see Fig. 4). The tentacleless body of the Hydra becomes slowly resolved into a dense spherical mass of coagulated protoplasm, in which no distinction between the individual cells can be made out, and remains in this condition as long as it is kept at a low temperature (see Fig. 3), but quickly forms tentacles and a double layer of cells again when it is re-

[1] Loeb: *American Journal of Physiology*, 1900. *IV.*, p. 178.

turned to the temperature of the room. Thus a lowering of the temperature seems to produce essentially the same effect on Hydra as the contact stimulus on the Campanularian Hydroid in Loeb's experiment. Likewise the structural changes appear to be identical with those produced by the low temperature upon the Protozoa.

Hydra react to variations in the temperature in another way which is interesting when compared to the reactions of Protozoa

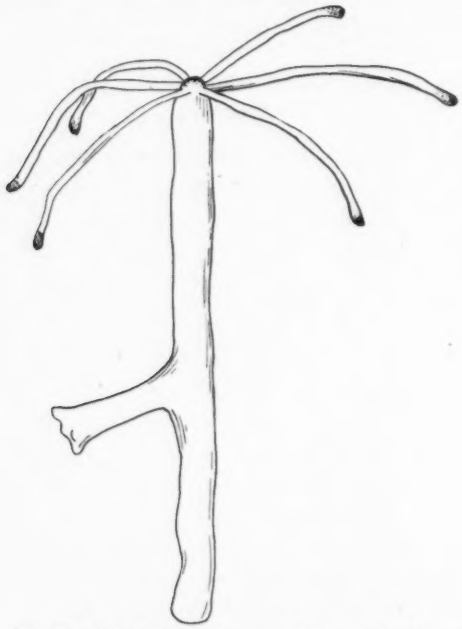


FIG. 1. A budding Hydra after an exposure of twenty-four hours to a temperature of 6°C. The body is slightly shortened and thickened, and the absorption of the ectoderm and entoderm cells has begun in the tips of the tentacles.

under the same conditions. It has been a fact of common observation that the rate of cell division varies directly with the temperature for all temperatures below the critical point. In my experiments on *Stentor* I showed that a lowering of the temperature not only inhibits cell division but brings about the reverse process. If a *Stentor* in the process of division be placed at a

temperature of 4°C. a fusion of the partially divided halves takes place. Among Hydra the formation of buds, which finally become distinct individuals, may be considered analogous to the process of cell division among Protozoa. It was found that if a Hydra in the earlier stages of the process of budding be placed

FIG. 2. The same Hydra as in Fig. 1, after an exposure of six days to a temperature of 6°C. The absorption of the tentacles and bud is nearly complete.

at a temperature of 4°C., not only does the growth of the bud stop instantly, but an absorption of the bud into the body of the parent commences, and continues until all traces of the bud have disappeared. (See Figs. 1 and 2.) In order to demonstrate this absorption of the bud, great care is needed in lowering the

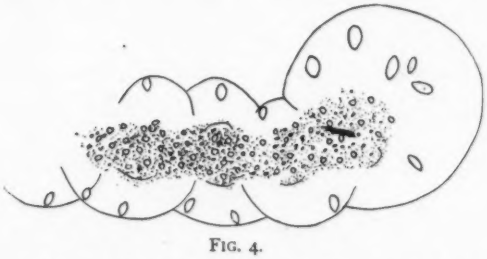


FIG. 4.

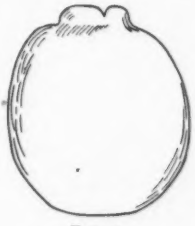


FIG. 3.

FIG. 3. The final resting stage of Hydra, formed after an exposure of four to seven days to a temperature of 6°C. The body consists of an undifferentiated mass of protoplasm.

FIG. 4. The tip of a tentacle of a Hydra that has been exposed to a temperature of 6°C. for twenty-four hours, showing the dissolution of the octoderm and endoderm cells.

temperature. If the temperature is quickly reduced to 1°C. the Hydra go to pieces, but if the temperature be maintained at from 4° to 6°C., and is not suddenly varied in either direction, the process of absorption can be easily seen. Six or seven days are required for the complete disappearance of the bud. These two experiments seem to show that among the Cœlenterates as well

as the Protozoa, a lowering of the temperature brings about a reversal of the vital phenomena and the formation of simple resting stages.

II. The Effect of Variations in the Temperature Upon Developmental Processes.

It has been frequently observed that eggs, spores, cysts, or other resting stages of the motile organism which are formed to tide over some unfavorable conditions in the life-history of the animal, will not develop into the motile form unless they are exposed to the very conditions that brought about their formation, and normally intervene before development commences. Thus Braem[1] found that the statoblasts of Bryozoa, and the winter eggs of *Apus* would develop only after they had been exposed to a certain degree of low temperature. In this case the physical change produced in the protoplasm of the egg or statoblast by the low temperature seems to be necessary before the developmental processes can originate.

Dr. Loeb suggested to me the possibility that the same thing might be true for the development and metamorphosis of the chrysalids of a common moth, *Cecropia*, that are formed in the Autumn, but do not complete the metamorphosis until the following Spring. To test this hypothesis and see if other means besides that of low temperature would suffice to start development, the following series of experiments was performed:

On October 15, 1901, before they had been exposed to any frosts, a large number of cocoons were brought into the laboratory. Many of these chrysalids were found to be parasitized by an ichneumon fly, and only a small number were available for the experiments. The cocoons were kept in the laboratory at a temperature of 20° C. until November 27. They were then divided into two lots. One lot was kept constantly at a temperature of 20° C., as a control series, and the other was placed outdoors for six days, at a time when the temperature fell below 0° C. each night. At the end of the six days, these cocoons were brought back into the laboratory, and kept with the others at a temperature of 20° C. On January 27, the chrysalids that had

[1] Braem, *Jahrber. Schles. Ges. f. nat. Cult.* (Zool. Bot. Sec.), 1895, p. 2.

been exposed to the low temperature began to produce moths, and all of them had completed the metamorphosis by February 3. None of the chrysalids that had been kept at a temperature of 20° C. showed any signs of development. Several of the cocoons were opened, but the chrysalids were in the same condition, as far as could be seen, as when they were collected.

This result indicated that a lowering of the temperature at least accelerates the metamorphosis of the chrysalids. To determine whether the effect of the low temperature on the larva consisted in an extraction of water from the protoplasms, as was the case in the low temperature experiments on the Protozoa, the experiment was now varied as follows: The cocoons, that had been kept constantly at a temperature of 20° C., were now, on February 3, divided into three lots. One lot was retained at the room temperature, 20°C., another lot was exposed outdoors to a temperature of about — 10° C. for seven days, and the third lot of four cocoons was placed in a desiccator over sulphuric acid for two days. These four cocoons, while in the desiccator, lost water as is shown by the following record of weights:

	Weight when Placed in Desiccator, Feb. 3.	Weight when Removed from Desiccator, Feb. 5.
1.	17.0555 g.	17.031 g.
2.	15.184 g.	15.173 g.
3.	16.630 g.	16.603 g.
4.	15.115 g.	15.095 g.

These four cocoons produced moths on March 4, 10, 13 and 14. On March 24, moths emerged from the cocoons of the second lot that had been exposed to the low temperature, but on March 26 the cocoons of the control series that had been kept continuously at the room temperature produced moths also, showing that this last exposure to a low temperature was too late to have any effect. The desiccation hastened the development by about two weeks. We see from this experiment that the original exposure to a low temperature in November, soon after the cocoons were first brought into the laboratory, hastened the development by two months, and that the desiccation within two months before all the cocoons produced moths sufficed to accelerate the development materially. These experiments are far from satisfactory because of lack of material, but they furnish

testimony to the conclusion already reached by Braem and others, that in resting stages of this sort, development can commence only after some physical change has occurred in the protoplasm through the action of a low temperature or other changes in the external conditions. These experiments further seem to show that the changes produced in the protoplasm by lowering the temperature are identical with those produced by an extraction of water, as has already been indicated in the experiments on Protozoa.

It is interesting to note that the same forms of stimuli (*i. e.*, a lowering of the temperature and an extraction of water), which hasten the development of the moth, also produce artificial parthenogenesis of the starfish egg. This fact lends weight to the idea, expressed by Loeb, that artificial parthenogenesis consists merely in the acceleration of developmental processes already present in the egg.

III. Effect of Temperature on the Absorption of Water by Muscle.

If the conclusion drawn from these earlier experiments, that a reduction of the temperature produces changes in the protoplasm that cause it to lose water is true, then variations in the temperature ought to have a decided effect on the absorption of water by muscle or other animal tissue. The experiments of Loeb[1] and Webster[2] on the gastrocnemius of the frog have demonstrated that this muscle always behaves in a very constant way, as far as can be determined by its change in weight, toward each salt solution in which it is immersed. In some salt solutions the muscle always absorbs a definite amount of water at the normal temperature, in others of the same osmotic pressure it always loses a definite amount. The only variation in this behavior of the muscle toward salt solutions occurs with the change of seasons, the muscle of winter frogs differing widely from summer frogs in this respect. This fact had been the only indication that temperature in any way affected the absorption of water by the frog's muscle, and the influence of the temperature

[1] Loeb, *Archiv. f. d. ges. Physiol.*, 1899, LXXV., p. 303.

[2] Webster, Univ. of Chicago Decennial Publications, 1902, X., p. 105.

alone was not clear in this case. In order to ascertain the influence of temperature upon this process and to obtain, if possible, some quantitative estimate of its action, I started a series of experiments to test the absorption of water by frog's muscle in the same solution at different temperatures.

All the salt solutions were used at dilutions isotonic with *m*/8 NaCl which is supposed to represent as nearly as possible the average osmotic pressure of the muscle. When tested at the normal temperature (20°C.), the solutions of all the salts experimented with, fall into one of three classes: first, those solutions which cause the muscle to absorb water as is shown by its increase in weight, for example, the univalent salts, KCl and NH_4Cl, and salts with a bivalent anion and two univalent kations as Na_2SO_4; second, those solutions which cause the muscle to lose water as shown by its decrease in weight, for example, salts with a bivalent kation and two univalent anions like $CaCl_2$ or or $SrCl_2$; third, those solutions which leave the water content of the muscle unaltered. LiCl is the best example of this third class. *m*/8 NaCl usually falls in this group, although in my experiments, I found that *m*/8 NaCl caused a slight increase in weight, and that *m*/6 NaCl was more nearly isotonic with the muscle.

The method used in the experiments was the same one that has been elaborated so successfully by Webster.[1] A large amount of each solution was made up isotonic with *m*/8 NaCl, and was then divided among dishes which were kept constantly at the following temperatures: 1°C., 20°C., 25°C., 27°C., 29°C., 31°C., 36°C., 38°C., 45°C. and 55°C. The gastrocnemius muscle of the frog was used in the experiment. The muscles were carefully weighed and then distributed among the dishes containing the solution to be tested at the temperatures named above. The muscles were weighed after remaining in the solutions for three hours, and again after twenty-four hours, and the gain or loss in the water content calculated in percentages of the original weight of the muscle.

The results of the experiments are given in Table I., in which are given curves showing the effect of temperature on the absorp-

[1] Webster, *loc. cit.*

tion or extraction of water after the twenty-four-hour exposure to each of the solutions. The curves for the three-hour exposure to the solutions are not given, because the twenty-four-hour

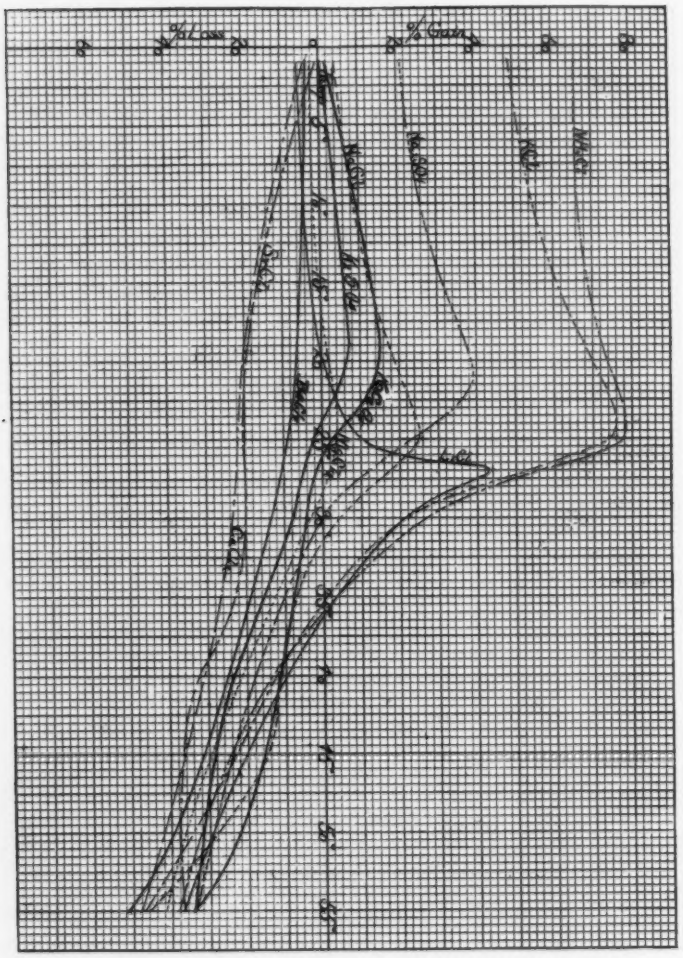


TABLE I. Curves showing the effect of temperature on the absorption of wat[illegible] by the gastroc-nemius muscle of the frog in various solutions isotonic with *m*/8 NaCl. The period of exposure to the solutions was twenty-four hours.

curves are entirely typical of the results obtained in both cases.

In considering the temperature effects we may classify them as regards their bearing on the absorption phenomena in the three classes of solutions mentioned above.

First, of those salts whose solutions, isotonic with *m*/8 NaCl, cause an absorption of water at the normal temperature (20°C.), the following were used: NaCl, KCl, NH_4Cl, Na_2SO_4, K_2SO_4 and $K_2C_2O_4$. In all of them, as is shown by the curves, the absorption of water varies directly with the temperature up to a certain critical point, in the neighborhood of 25°C., at which a sudden loss of water commences which increases rapidly with a further elevation of temperature. The form of the curve is the same for all the solutions, regardless of whether the initial absorption is great or small, and at about 50°C. the loss of water becomes practically the same in all the solutions. The form of these curves is strikingly like that showing the direct effect of temperature upon the amount of water in protoplasm, independently of the specific action of any salt solution, as is shown by the curve for *m*/8 NaCl which approximates as nearly as possible the fluid which normally bathes the muscle during life, as far as its chemical composition is concerned. For these reasons it seems probable that the effect of temperature upon the absorption of water in these solutions is due to the physical changes induced by the variations in the temperature in the protoplasm of the muscle. The rise in temperature may also accelerate the specific chemical action of the solution upon the muscle proteids, but in any event this only increases the result produced by the temperature alone. The amount of water in the protoplasm of a Protozoan varies directly with the temperature up to the critical point which marks the beginning of heat rigor, and it is interesting to find that the same thing occurs in muscle when immersed in solutions which are isotonic with its own substance. Above the critical point the heat rigor causes the same loss of water in all the solutions regardless of their chemical composition.

The same temperature effects are still better shown in curves of the absorption in solutions of the second class, *i. e.*, those which cause neither a gain or loss of water in the muscle at the normal temperature. Of the solutions of these salts, three were

used: LiCl, $MgCl_2$ and $m/6$ NaCl. Usually $MgCl_2$ has been described to act like $CaCl_2$, $BaCl_2$ and $SrCl_2$, in whose class it would naturally fall, in causing a loss of water. But although I tested its action many times, in all my experiments it had practically no effect on the weight of the muscle at the room temperature. It will be seen by examining the curves for LiCl and $MgCl_2$, that at a temperature of 1° C., the muscle loses a small amount of water. This loss of water decreases steadily as the temperature is raised until just above 20° C., an absorption of water commences, which increases until the critical point is reached. Above this point the muscles lose water very rapidly just as in the other solutions. Thus in these solutions, which appear to have no effect on the muscle at the normal temperature, there is a loss of water at low temperatures, a gain at temperatures between the normal and the critical point, and a very rapid loss above the critical point, which is exactly the effect that changes in temperature have been shown to have on the protoplasm of the cells of Protozoa, when only the physical condition of the protoplasm is modified by the variations in the temperature. $m/8$ NaCl also should have no effect on the amount of water in muscle at the room temperature, but in many cases, especially with the muscles of winter frogs, this solution appears to be hypotonic to the muscle substance. As Webster has shown, the osmotic pressure of the muscle varies with the season of the year, being higher during the winter, which is the condition we should expect from the observed effect of low temperatures on protoplasm. In my experiments (with winter frogs), $m/8$ NaCl invariably caused a slight gain in weight, but $m/6$ NaCl was found to be isotonic with the muscle, and the curve for this solution corresponds exactly with that for LiCl. Thus in all these solutions which appear to have no chemical effect on the muscle, as far as can be determined by the changes in weight, the amount of water in the muscle varies directly with the temperature up to the critical point, and inversely with the temperature above that point, and it is reasonable to suppose that the same thing occurs in the muscle in its normal surroundings within the body.

The curves for $CaCl_2$, $BaCl_2$ and $SrCl_2$ are very different from

those of the first two classes of salts. Solutions of these salts, isotonic with *m*/8 NaCl, cause a loss in weight of about 20 per cent. at a temperature of 20° C. It will be seen from the curves that in each of these solutions the loss of water is very slight at a temperature of 1° C., and that the decrease in weight increases steadily as the temperature is raised. But at a temperature of 50° the physical changes in the protoplasm overbalance the specific action of any solution and the muscles lose practically the same amount of water in all solutions. It appears that in the case of these solutions we are dealing with specific ion effects, as Loeb[1] has already suggested, and that the curve may be interpreted as follows: The speed of any chemical combination varies directly with the temperature. At a temperature of 1°C. the reaction between Ca, Ba or Sr and the muscle proteids is so greatly slowed that the solution has no effect on the muscle, and the small loss of water is due entirely to the physical changes in the muscle produced by the low temperature, as in LiCl, *m*/6 NaCl, and the other solutions which have no specific action on the muscle substance. As the temperature is increased, the reaction between the muscle proteids and the ions is accelerated, and this chemical action of the Ca, Ba or Sr ion overcomes the effect of the physical changes produced by the temperature, and the loss of water steadily increases because these ion proteid compounds, like Ca-soaps, hold very little water. It is worthy of notice, however, that at about 25° C. there is a break in the continuity of the curves, corresponding with the rapid absorption of water in the other solutions, which indicates a change in the physical condition of the protoplasm that neutralizes temporarily the specific ion effects.

In distilled water the amount of absorption by the muscle is decreased by lowering the temperature, as is shown by the following result for a one-hour exposure to distilled water: Percentage of absorption

at 2° C.	40.7
at 30° C.	53.1

[1] Loeb, *loc. cit.*

SUMMARY.

1. In Hydra as well as Protozoa, a lowering of the temperature brings about certain definite structural changes that result in the formation of an undifferentiated resting stage.

2. The inhibition of cell division and reversal of vital phenomena by a reduction of the temperature is shown in Hydra by the fact that at a temperature of 6° C., the growth of a new bud ceases, and the partially formed bud is gradually absorbed into the body of the parent animal.

3. A lowering of the temperature and an extraction of water both bring about the same physical changes in the protoplasm which serve to accelerate the development and metamorphosis of the chrysalids of *Cecropia*.

4. The absorption of water by the gastrocnemius muscle of the frog in those salt solutions which, when used at dilutions isotonic with its own substance, either have no chemical effect on the muscle at the room temperature, or cause an increase in weight, varies directly with the temperature, until the critical point is reached at which the muscle proteids begin to coagulate. In solutions of the same osmotic pressure, which cause the muscle to lose water at the room temperature, this loss of water varies directly with the temperature. Above the critical point of temperature the muscles lose practically the same amount of water in all solutions, regardless of their initial effect on the muscle.

ZOOLOGICAL LABORATORY,
WASHINGTON UNIVERSITY, March 18, 1903.

THE EMBRYONIC DEVELOPMENT OF THE OVARY AND TESTIS OF THE MAMMALIA. (PRELIMINARY ACCOUNT.)

BENNET M. ALLEN.

The following is a preliminary account of work begun in the spring of 1900, upon the subject of the development of the ovary and testis of the mammalia. The rabbit and pig have served as the subjects for this work. The results will here be very briefly set forth, a more detailed account being reserved for a later paper. The material studied includes various stages in the development of the ovary and testis of the rabbit, from the thirteen-day embryo to and including adult stages. The pig material includes only embryonic stages, but is more complete for the period covered than is the rabbit material.

The work was carried on with the aim of solving the following problems: (1) the origin and development of the seminiferous tubules and their homologues in the ovary; (2) the origin, development, and homologies of the rete tubules, also their relations to the Malpighian corpuscles of the mesonephros on the one hand, and to the seminiferous tubules of the testis and medullary cords of the ovary on the other; (3) the origin, development and homologies of the connective tissue elements and interstitial cells of the ovary and testis.

Incidental to the solution of these problems, the work has involved to a greater or less extent, a consideration of the following allied problems: (1) the development of sex cells; (2) the morphological phases of sex differentiation; (3) cell degeneration in the sex gland and rete region, (4) the degeneration of the mesonephros, and the development of the Wolffian and Müllerian ducts.

The results of work in so large a field can be, only to a limited extent, new. Certain of the following results are confirmatory of the work of other authors, to whose results I shall refer in a later paper. The very contradictory opinions met with in the literature on the subject call for confirmatory evidence upon these problems.

The earliest rudiment of the sex gland is situated in the genital ridge, which consists of a zone of thickened peritoneum, running the entire length of the mesonephros, parallel and close to the mesentery which unites the latter to the body wall. The rete is formed from the anterior part of the genital ridge and extends, in the pig, from about the region of the sixth glomerulus to a point about opposite the twentieth glomerulus, as shown in a number of series. However, these limits are variable. In the rabbit the limits are more constant, but still variable, the rete extending approximately from the sixth to the twelfth glomerulus. The anterior end of the sex gland rudiment slightly overlaps the posterior end of the rete region. Posterior to the sex gland is a section of the genital ridge that does not develop beyond the very early formation of a region of thickened peritoneum. In each of these three zones of the genital ridge are found scattered cells with distinct cell walls, clear cytoplasm, large round nucleus, centrosphere and centrosome — the so-called primitive sex cells.

In its early stages of development the genital ridge consists of a thickened layer of peritoneum overlying a loose mesenchyma; the cells of the latter are to all appearances identical with those of the peritoneum, from which they undoubtedly originate. This resemblance applies both to the character of their nuclei and to their lack of definite cell boundaries. The peritoneal layer and underlying mesenchyma are separated from one another by the basement membrane of the former. This is formed by the interlacing of protoplasmic fibrils given off by the cells of both layers. In later stages, one finds a progressive crowding of the peritoneal nuclei. In the rete and sex gland regions this results in the formation of tubular peritoneal invaginations, which are limited from the surrounding mesenchyma by the persistent membrana propria. In the sex gland region these tubular cords of cells may be known as the sex-cords. At this indifferent stage they are closely massed together side by side, and the very narrow interspaces between them contain scattered mesenchyme cells, which from now on, may be considered under the general term of stroma. This is used to designate the loose connective tissue of both ovary and testis. Invaginations, in all respects similar to these sex-cords, arise from the peritoneum of the rete region. They lie further

apart than do the sex-cords, and penetrate more deeply into the mesenchyme. Both rete-cords and sex-cords are at this stage devoid of a lumen. In cross-section they show a limiting membrana propria, within which is a single layer of cells arranged with their bases attached to the membrana propria and their apices meeting at a common point in the center — the rudiment of the lumen.

The mesentery of the sex gland is formed by the proliferation of connective tissue cells from the peritoneum, in areas of restricted width immediately ventral and dorsal to the sex gland. The ventral area is by far the more important of the two sources. The albuginea is in large part formed from the cells comprising the proximal parts of the sex-cords. They are formed at the time when the sex gland has begun to assume definite shape. At this time the rapidly dividing cells of the attached ends of the sex-cords have become differentiated from those of the more distal portions in that they elongate, become smaller, and acquire the property of staining more deeply. They then break away from the peritoneum on the one hand, and from the sex-cords on the other. They may still for some time be found attached to portions of the membrana propria that ensheathed them. They become mingled with certain exactly similar intertubular mesenchymal elements, to form the albuginea, which is essentially one with the remaining connective tissue or stroma of the sex gland. Sexual differentiation is first manifested by the cessation of growth of the sex-cords of the ovary. We can then distinguish them as medullary-cords. The peritoneum of the ovary begins to increase in thickness, and eventually forms the cortex of the adult ovary in a manner to be briefly indicated below. The albuginea of the ovary forms a broader, looser, and more irregular layer than does that of the testis. In the testis the peritoneum ceases to grow, in large measure at least, its cells becoming flattened, and in later life practically disappearing. The rete cords grow backwards from their points of origin, and enter the anterior part of the sex gland. They branch and anastomose throughout their course, sending branches to the Malpighian corpuscles on the one hand, and on the other to the seminiferous tubules of the testis. The branches passing to the Malpighian corpuscles

meet evaginations from the capsules of Bowman, with which they fuse. Such evaginations are irregular in number, as many as three having been counted upon the same glomerulus. Some glomeruli may send out none at all. The tubuli recti connecting the rete-cords with the seminiferous tubules are likewise irregular in number, being apparently called forth wherever needed.

Seminiferous tubules, medullary-cords, and rete-cords are homologous structures. Not only are they of similar origin, but their component cells show similarities. Two kinds of cells are found in all three structures: (1) primitive sex cells, which have already been described; (2) cells more or less variable but agreeing with one another in not having clearly marked cell limits, and also in the absence of centrosphere and centrosome. The cells of this second class form the germinative cells of the seminiferous tubules, the follicular cells of the medullary-cords, and the rete cells proper of the rete-tubules.

Returning to the subject of the ovary, the peritoneum at the time of separation of the medullary-cords, contains no differentiated sex cells. Such may exist, but they are at all events indistinguishable from the remaining peritoneal cells. The peritoneum or germinal epithelium, as it may now be termed, next begins to give rise to the cords of Pflüger, which branch and anastomose in a similar manner to the medullary-cords. Some of these cords of Pflüger may contain a well-defined lumen, in the case of advanced embryos of the pig (15 cm. length). In these later stages the inner ends of the cords are broken up to form follicles. Follicles are likewise formed in medullary-cords. These however, have never more than one layer of follicular cells in the forms studied.

The rete-cords come in contact with the medullary-cords, and are then scarcely distinguishable from the latter in the case of the rabbit. They contain no sex cells in later stages of the ovary of that animal, although such are present in the rete tissue when it is first laid down. By this criterion alone can one, in a very general way, distinguish between medullary-cords and rete-tissue lying within the rabbit's ovary. In the pig, on the other hand, the rete-tissue shows some very interesting characteristics. The portions of the rete-cords lying within the

ovary undergo similar differentiation to the medullary-cords and the cords of Pflüger forming the cortex. The rete-tissue within the ovary of the 18 cm. pig embryo is found to contain young follicles, each with a single layer of follicular cells; the enlarged oöcyte in the center having passed through the synapsis condition, characteristic of one stage in the development of the young oöcytes. All such follicles subsequently degenerate. In the testis the intra-glandular portions of the rete-tubules are similar to the seminiferous-tubules, but differ from them in their much smaller diameter and in the earlier acquisition of a lumen. They contain the sex cells characteristic of the seminiferous-tubules. These are at first present in the extra-glandular region of both ovary and testis, but disappear more or less completely in later stages. No attempt was made to study out the fate of the sex cells of the rete-tubules of the pig testis. They are still present in the 25 cm. pig embryo. In the rabbit they are found in the rete of the testis twenty-four days after birth, but are not to be found in that of a rabbit killed 140 days after birth. The rete-tubules are so completely united by anastomosis that their connected lumen forms a large irregular cavity divided here and there by irregular partitions formed by the walls of the several rete-tubules.

The connective tissue elements of ovary and testis are derived from the peritoneum. In early stages they are not distinguishable from the cells that make up the sex-cords, except that the latter are marked off from the stroma by their membrana propria. As before stated, the albuginea is largely formed by actual transformation of the basal part of the sex cords into connective tissue elements.

The interstitial cells are characterized by a large nucleus, distinct cell boundaries, a centrosphere and centrosome, and very granular cytoplasm. They first appear in the stroma of both testis and ovary of the pig of 2.5 cm. length. They are far more numerous in the testis than in the ovary. Their appearance is coincident with that of a large number of fatty globules in the peritoneum and sex cords. In the testis they persist for a long time. In the ovary, however, the few cells appearing at this stage speedily disappear. In both organs they divide by mitosis.

This process soon ceases in the ovary, while in the *testis*, on the other hand, division figures are found in the interstitial cells at a stage as late as the 7.5 cm. embryo. In the testis of the 15 cm. embryo, they have begun to degenerate. This process manifests itself in a shrinkage of the cytoplasm. Interstitial cells first form in the testis of the rabbit embryo of a stage between seventeen and twenty-one days. They are found to be still dividing by mitosis eight days after birth. They are very rare, however, in the stage of twenty-four days after birth.

No interstitial cells were found in the *ovary* of the embryo rabbit, they being first met with in females killed forty-five days after birth. Here they are scarce, but unmistakable. Considerable light is thrown upon their origin by a study of the eighty-five-day rabbit. In the ovary of this stage they are very common, their origin from the cells of the theca interna of atretic follicles being clearly shown. This, taken in connection with the additional fact that they make their appearance in the 2.5 cm. pig embryo coincident with the fatty degeneration of the germinative cells of the seminiferous tubules and their ovarian homologues, together with that of many cells of the germinal epithelium, would lead us to conclude that cell degeneration offers the stimulus or condition that brings about the formation of the interstitial cells.

Interstitial cells do not develop from unmodified connective tissue cells, such as those comprising the theca externa and the general ovarian stroma. Such stroma cells must be transformed into cells of the theca-interna by the direct or indirect influence of the growing follicles, before they are again susceptible to the influences exerted by the process of cell degeneration. Atresia of small follicles that are not surrounded by a theca-interna does not bring about the formation of interstitial cells. Many such small follicles are found to degenerate early and late in the history of the ovary.

No evidence has been found favoring the theory of the early segregation of the sex cells, but I am not prepared to say that my work has in any way tended to disprove such a theory. Sex cells appear in the very youngest stages studied (pig embryo, 0.6 cm. length and rabbit embryo of 13 days' age). They

are most common in the region where the sex-gland will eventually form, occurring both in the peritoneum and among scattered subperitoneal cells of mesenchymal nature. They are prominent in the sex-cords of a later stage. In the 1.8 cm. pig embryo, immediately after the separation of the sex-cords from the peritoneum, the latter is found to contain no sex-cells distinguishable as such. If the sex-gland be an ovary, they soon (2.5 cm. pig embryo) make their appearance in the peritoneum, and especially in the cords of Pflüger growing inward from it. These cords of Pflüger increase by growth at their bases, *i. e.*, their points of connection with the peritoneum. Hence there is a continual development of peritoneal cells to form the primitive ova distinguishable as such. The case of the seminiferous tubules is not so clear. Well-developed sex cells are found in them from the start. On the other hand, all stages of transition are found to link the germinative cells with the sex cells. These transitional cells are found in the testis of the pig at as late a stage as the 13 cm. embryo.

Whether the sex-cells that appear in the very early stages of embryonic development ever produce functional sex products in the testis, is a question that cannot easily be solved in this form. Certain it is, however, that true sex products do form in both ovary and testis from apparently undifferentiated cells of peritoneal origin, and that those which are functional in the ovary form *exclusively* from this source. The sex-glands and rete tissue are the seat of extensive processes of cell degeneration. I shall not here enter upon a discussion of the different forms which this process assumes, but shall defer treatment of these considerations to the more complete account.

This piece of research has brought up many interesting facts, bearing upon questions touching upon the action of trophic stimuli in embryonic development. Perhaps the most striking example of this is the formation of follicles in that portion of the rete tissue lying within the ovary. The extra-ovarian part, or that remaining in the mesonephros, does not contain follicles, although it is of precisely the same origin as the intra-ovarian portion. The influence of the ovary reaches out a short distance into the mesonephros, as can be seen by the presence there of a

few sex cells, which are more numerous in the regions nearest to the ovary. There is a definite interaction between the capsules of Bowman and the rete-cords lying nearest to them. Each sends forth a process to meet the other. In the testis the rete cords send out processes (tubuli recti) to meet the seminiferous tubules at their inner ends. Each tubule receives its rete branch. A large number of tubuli recti can arise, from a single rete-tubule at different points in its course. The connection between cell degeneration and the formation of interstitial cells has already been discussed. The uriniferous tubules of those glomeruli with which the rete-cords come in contact persist as the rete efferentia of the male, while the remaining ones disappear wholly, or in large part at least. A few rudiments of these rete efferentia tubules persist in contact with the rete ovarii. Such rudiments are very rare in the pig embryo of 25 cm. length.

HULL ZOOLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, April 20, 1903.